University of Alberta

Post-surgical electrical stimulation facilitates axonal regeneration in carpal tunnel syndrome

by

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Abstract

Successful axonal regeneration and functional recovery does not always occur following peripheral nerve injuries. Electrical stimulation (ES) is a potentially useful clinical tool for improving axonal regeneration. In a randomized controlled study we investigated the effectiveness of the application of 1 h of post-surgical ES on axonal regeneration and functional outcome in subjects with extensive axonal loss due to moderate and severe carpal tunnel syndrome. ES was found to be a promising adjunct clinical tool for treatment. Equally important, it was well tolerated by the subjects and resulted in no complications. Two other studies examined if the additional use of a long pulse width stimulus during multiple point stimulation (MPS), a key outcome measure for the ES study, would increase the accuracy of motor unit number estimates and improve test-retest reliability. The modified MPS was more accurate and had higher test-retest reliability than the standard MPS in young subjects.

Table of Contents

Chapter 1.	**NERVE COMPRESSION, AXONAL REGENERATION, AND FUNCTIONAL OUTCOME**	
1 – I.	Introduction	1
1 – II.	NERVE ANATOMY	4
1.	Peripheral nerves	4
2.	Nerve fibers	4
3.	Topography of peripheral nerves	5
4.	Connective tissues	5
5.	Axoplasmic transport	7
6.	Blood supply to the peripheral nerve	9
7.	Blood-nerve barrier	10
1 – III.	NERVE COMPRESSION	11
1.	Pathophysiology of nerve compression	12
2.	Degrees of nerve injury	12
1 – IV.	REGENERATIVE RESPONSE	14
1.	Distal nerve stump	15
2.	Soma	16
3.	Axonal regeneration	18
4.	Rate of axonal regeneration	19
1 – V.	NERVE COMPRESSION WITHIN THE CARPAL TUNNEL	20
1.	Median nerve	20
2.	Anatomy of the carpal tunnel	22
3.	Etiology of carpal tunnel syndrome	23
4.	Symptoms and signs of carpal tunnel syndrome	25

5.	Clinical correlation to nerve compression	26
1 – VI.	ELECTRODIAGNOSIS OF CARPAL TUNNEL SYNDROME	27
1.	Median nerve conduction studies	28
2.	Motor unit number estimation (MUNE)	31
3.	Treatment for carpal tunnel syndrome	34
4.	Electrical stimulation	35
1 – VII.	THESIS	40
1.	Objectives	40
2.	Hypotheses to be tested	40
3.	Rationale	41
References		44

Chapter 2.	**IMPROVING THE ACCURACY OF THE MULTIPLE POINT STIMULATION TECHNIQUE**	
2 – I.	Introduction	66
2 – II.	Methods	66
1.	Subjects	66
2.	Motor unit number estimation	67
2 – III.	Results	68
2 – IV.	Discussion	69
References		72

Chapter 3.	**TEST-RETEST RELIABILITY OF A MODIFIED MULITPLE POINT STIMULATION TECHNIQUE FOR MOTOR UNIT NUMBER ESTIMATION**	
3 – I.	Introduction	78
3 – II.	Methods	79
1.	Subjects	79
2.	Motor unit number estimation	79
3 – III.	Results	81
1.	Sample size and potential sampling bias	81
2.	Motor unit number estimation results and test-retest reliability	82
3 – IV.	Discussion	83
References		86
Chapter 4.	**POST-SURGICAL ELECTRICAL STIMULATION FACILITATES AXONAL REGERNATION IN CARPAL TUNNEL SYNDROME**	
4 – I.	Introduction	92
4 – II.	Methods	95
1.	Overview of methods	95
2.	Subjects	95
3.	Nerve conduction studies	96
4.	Treatment	97
5.	Surgical procedure	98
6.	Electrical stimulation of the median nerve	99
4 – III.	Outcome measures	99
1.	Motor unit number estimation	100

2.	Median nerve conduction studies	102
3.	Levine self-assessment questionnaire	102
4.	Semmes Weinstein monofilaments test	103
5.	The Purdue pegboard test	105
6.	The Moberg pick-up test	106
4 – IV.	Statistical Analyses	107
4 – V.	Results	108
4 – VI.	Outcome Measures	108
1.	Motor unit number estimation	109
2.	Median nerve conduction studies	109
3.	Levine self-assessment questionnaire	111
4.	Semmes Weinstein monofilaments test	112
5.	The Purdue pegboard test	112
6.	The Moberg pick-up test	113
4 – VII.	Discussion	115
1.	Effectiveness of electrical stimulation on axonal injury	115
a.	Motor axonal regeneration	115
b.	Sensory axonal regeneration	118
2.	Clinical implications	119
3.	Fraction of motor neuron pool stimulated for electrical stimulation effects	120
4 – VIII.	Potential mechanisms of action of electrical stimulation	120
4 – X.	Limitations and future directions	123
1.	Subject	123
2.	Experimental procedures	123

References 124

Chapter 5. CONCLUSION AND FUTURE DIRECTIONS

5 – I. Improving the Reliability of the Multiple Point Stimulation Technique 154

5 – II. Effectiveness of electrical stimulation in enhancing axonal regeneration and functional outcome 155

5 – III. Effectiveness of using a longer pulse width to improve multiple point stimulation 156

5 – IV. Future directions for the effectiveness of electrical stimulation 156

References 158

Appendix 1 Levine self-assessment questionnaire scales 159

List of Tables

1 – 1.	Comparison of the Seddon and Sunderland classification system for nerve injuries	57
1 – 2.	Carpal tunnel syndrome severity based upon standard nerve conduction study criteria	58
2 – 1.	Number of surface motor unit action potentials collected at 0.05 ms, 1 ms, and in total for each subject	73
3 – 1.	Comparisons of the number of surface motor unit action potentials (SMUPs) collected with the standard and modified multiple point stimulation techniques during test and retest conducted on separate days	88
3 -2.	Comparisons of the mean surface motor unit action potential (SMUP) negative-peak amplitude, and motor unit number estimation (MUNE) during test and retest conducted on separate days	89
4 – 1.	The calibration (i.e., markings) of the Semmes Weinstein monofilaments, force applied, and degree of severity in sensory perception loss	135
4 – 2.	The number of symptomatic hands included in each outcome measure at baseline and at each post-op	136
4 – 3.	Median values of the outcome measures in the treatment group at baseline and at each post-op	137
4 – 4.	Median values of the outcome measures in the control group at baseline and at each post-op	138

List of Figures

1 – 1.	Normal anatomy of nerves.	59
1 – 2.	Peripheral axonal regeneration	60
1 – 3.	Chromatolysis: reaction of perikaryon to injury of its axonal extension	61
1 – 4.	Median nerve course and neural branching in the extremity	62
1 – 5.	Median nerve distribution	63
1 – 6.	Cross-sectional view of the carpal tunnel and its contents at the level of the first row of carpal bones	64
2 – 1.	Original surface motor unit action potentials obtained in a representative subject with stimulus pulse width durations of 0.05 ms (A) and 1 ms (B)	74
2 – 2.	Typical distribution of the sizes of the surface motor unit action potentials collected at the narrow pulse width (0.05 ms) (A) and wide pulse width (1 ms) (B) in a representative subject	75
2 – 3.	The distribution of surface motor unit action potentials collected with stimulation durations of 0.05 ms (A) and 1 ms (B) from all 10 subjects	76
3 – 1.	Reproducibility of the motor unit number estimation (MUNE), derived from the standard and modified multiple point stimulation (MPS) techniques, for each young subject	90
3 – 2.	Percentage errors of motor unit number estimation (MUNE) obtained when progressively smaller numbers of surface motor unit action potentials (SMUPs) were used for the estimates	91

4 – 1.	Experimental set-up. Set-up of electrodes during electrical stimulation	139
4 – 2.	Set-up of the multiple point stimulation technique	140
4 – 3.	The 12 objects that comprise the Moberg pick-up test (A) and a subject performing the test with his eyes closed (B)	141
4 – 4.	Comparison of motor unit number estimation in the control and treatment groups	142
4 – 5.	Sensory nerve conduction velocity	143
4 – 6.	Sensory nerve action potential amplitude (SNAP)	144
4 – 7.	Terminal motor latency	145
4 – 8.	Compound muscle action potential amplitude	146
4 – 9.	Comparison of Levine's Self-Assessment Questionnaire	147
4 – 10.	Comparison of the performance on the Purdue Pegboard Test using the treated hand	148
4 – 11.	Comparison of the performance on the Purdue Pegboard Test using the untreated hand	149
4 – 12.	Performance on the Moberg Pickup Test using the treated hand with eyes open	150
4 – 13.	Performance on the Moberg Pickup Test using the treated hand with eyes closed	151
4 – 14.	Performance on the Moberg Pickup Test using the untreated hand with eyes open	152
4 – 15.	Performance on the Moberg Pickup Test using the untreated hand with eyes closed	153

List of Abbreviations

Abbreviation	Definition
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CMAP	Compound muscle action potential
CNS	Central nervous system
CTS	Carpal tunnel syndrome
CV	Conduction velocity
DC	Direct current
DRG	Dorsal root ganglion
EMG	Electromyography
ES	Electrical stimulation
GAP	Growth-associated protein
Levine	Levine self-assessment questionnaire
Moberg	Moberg Pick-up test
MU	Motor unit
MUNE	Motor unit number estimation
MPS	Multiple point stimulation
NC	Nerve conduction
NFM	Medium-molecular weight neurofilament protein
NT-4/5	Neurofilament-4/5
PMR	Preferential motor reinnervation
PNS	Peripheral nervous system
Post-op	Post-operative

Purdue	Purdue pegboard test
SC	Schwann cell
SFI	Sciatic functional index
SNAP	Sensory nerve action potential
SMUP	Surface motor unit potential
SWM	Semmes Weinstein monofilament test
TCL	Transverse carpal ligament
TML	Terminal motor latency
WD	Wallerian degeneration

CHAPTER 1

NERVE COMPRESSION, AXONAL REGENERATION, AND FUNCTIONAL OUTCOME

1 – I. Introduction:

The peripheral nervous system (PNS) is capable of providing a permissive environment for axonal regeneration following peripheral nerve injuries (Kiernan, 1979). The injured axons must regenerate and reinnervate appropriate end targets in a timely manner for a chance at successful functional outcome. However, the permissive milieu of the PNS should not give the illusion that successful axonal regeneration and optimal functional outcome always ensue (Seckel, 1990; Yin *et al.*, 1998; Hall, 2005). Even after advancement in surgical techniques, the lack or incomplete recovery of sensory and motor functions following severe peripheral nerve injuries still remain major clinical problems (English, 2005; Johnson *et al.*, 2005). Indeed, in those situations, functional recovery rarely returns to the pre-injury level (Rosenbaum & Ochoa, 1993). Potential barriers for achieving successful axonal regeneration and functional recovery include: delay in axonal growth, misdirection of axonal sprouts at the injury site, growth to inappropriate end targets, (Sisken *et al.*, 1993) and the decline in the availability over time of essential components required to support axonal regeneration, such as Schwann cells and neurotrophins (Fu & Gordon, 1997).

To overcome the potential limitations of axonal regeneration and improve functional outcome following peripheral nerve injury, research has shifted from examining the underlying mechanical mechanisms to understanding the biological aspects of peripheral axonal regeneration (Lundborg, 2003). The suboptimal results following surgery suggest that peripheral nerve injuries should be treated not only at the physiological level, but also at the molecular level (Hall, 2005). This type of treatment is anticipated to enhance axonal regeneration and improve functional outcome. However, so far no such treatment is clinically applicable or available (Ahlborn *et al.*, 2007).

Application of electrical stimulation (ES) to a peripheral nerve is a promising clinical treatment for promoting the regenerative process and ameliorating functional recovery of injured peripheral nerves due to the promising results of ES in past studies on rats (Pomeranz *et al.*, 1984; Pockett & Gavin,1985; Roman *et al.*, 1987; Politis *et al.*, 1988; Al Majed *et al.*, 2000; Brushart *et al.*, 2002; Mendonca *et al.*, 2003; Geremia *et al.*, 2007), mice (Ahlborn *et al.*, 2007), canines (Rozman *et al.*, 2000), rabbits (Nix & Hopf, 1983), and humans (Amirjani, 2005). These studies have demonstrated that weak ES significantly promotes axonal regeneration and some early functional recovery following crushed, compressed and axotomized (transected) peripheral nerves.

Amirjani reported that ES promotes axonal regeneration, improves recovery, but does not enhance functional outcome in human subjects with moderate or severe carpal tunnel syndrome (CTS; Amirjani, 2005). These subjects had substantial axonal loss due to compression of the median nerve within the strict anatomical boundaries of the carpal tunnel. ES was applied for 1 h to the compressed median nerves immediately following carpal tunnel decompression surgery. The ES resulted in a significant enhancement in motor axonal regeneration, some early recovery, but marginal improvement in hand function (Amirjani, 2005). These suboptimal results might be due to a sodium channel blockade at the wrist (caused by local anesthesia applied during surgery), which could have prevented the depolarization of all the peripheral nerve fibers.

In our study moderate or severe CTS with substantial axonal loss will be used as a human model of axonal degeneration. The effectiveness of ES on axonal regeneration, functional recovery and outcome will be evaluated. ES will be applied to the median nerve, proximal to the injury site (i.e., away from the sodium channel blockade), which is postulated to produce even further significant improvement in axonal regeneration and enhance functional recovery and outcome.

CTS is the most common peripheral nerve injury (Werner & Andary, 2002), afflicting approximately 3.7% of the general adult population in the United States (Papanicolaou *et al.*, 2001). It is caused by the compression of the median nerve as it travels beneath the transverse carpal ligament (TCL) at the wrist. Compression can profoundly damage the sensory and motor fibers of the median nerve, subsequently impairing both the sensory and motor functions of the hand, and in turn, adversely affect an individual's ability to work and to participate in recreational activities (Sesto *et al.*, 2003).

Besides these adverse consequences, CTS also has economic implications. The direct medical expenses caused by CTS exceed 2 billion dollars US annually (Bitar *et al.*, 2002). To alleviate the median nerve compression that causes these deleterious effects, CTS decompression surgery is typically performed (see section I-VI-3; Franzblau & Werner, 1999). However, as aforementioned there is no surgical technique that ensures full functional recovery (Lundborg, 2003) and many individuals go on to long-term disability or change careers because of permanent residual pain attributed to CTS (Manktelow *et al.*, 2004). Furthermore, other high financial costs to society are attributed to the expenses of rehabilitation and the loss of productivity as a result of disability leave. Due to the reduction in quality of life of individuals with CTS and the economic implications, it is important to explore any treatments that may improve axonal regeneration and functional outcome in these individuals. At the present time, there are no clinically applicable treatments. One potential treatment, which has been shown to enhance axonal regeneration, is ES because it is tolerable and relatively non-invasive in human subjects (Amirjani, 2005).

This chapter will provide background information required to understand peripheral nerves, peripheral nerve compression, CTS, peripheral axonal regeneration, and the potential clinical applicability of ES to enhance axonal regeneration, functional recovery and outcome in individuals with moderate and severe CTS. Since motor unit number estimation is an important outcome measure used to quantify the severity of motor axon loss, efforts were made to

maximize the reliability of the method. This is of paramount importance when evaluating the effectiveness of new treatment.

1 – II. Nerve Anatomy:

1. Peripheral Nerves:

Peripheral nerves extend outside the central nervous system (CNS), which consist of the brain and spinal cord. The primary function of peripheral nerves is to provide communication to and from the CNS and the rest of the body via transmitted electrical signals (i.e., action potentials; 2008). The peripheral nerves do so without the protection of bones, consequently making them susceptible to mechanical injuries. For example, the median nerve is a peripheral nerve that can become compressed as it travels through the carpal tunnel at the wrist, resulting in sensory and motor deficits in the hand (i.e., CTS).

2. Nerve Fibers:

Nerve compression can severely affect the main functional components of peripheral nerves: the nerve fibers. Nerve fibers are comprised of axons and cytoplasm (axoplasm), which are enclosed by a membrane (axolemma) and further enclosed by a Schwann cell (SC) myelin sheath (Maggi *et al.*, 2003). Nerve fibers can be sensory, motor, or autonomic (Landers & Altenburger, 2003; Stewart, 2003; Delfiner, 1996). The cell bodies (somas) of the sensory, motor, and autonomic nerve fibers reside in the dorsal root ganglion (DRG), the ventral horn of the spinal cord and various autonomic ganglia, respectively (Delfiner, 1996).

Nerve fibers extend peripherally from a soma and are either unmyelinated or myelinated (Stewart, 2000). Unmyelinated nerve fibers are composed of several axons, which are associated with a single SC (Maggi *et al.*, 2003). In contrast, myelinated nerve fibers are enwrapped by a series of SCs arranged longitudinally along the length of a single axon, with each SC covering an axonal segment (Maggi *et al.*, 2003; Mackinnon, 2002; Donoff, 1995).The

myelinated segments of the axons aid the propagation of action potentials by acting as an insulator that helps to prevent transverse current leakage (Maggi *et al.*, 2003). In these myelinated axons the axolemma is exposed at a gap between adjacent SCs, known as the node of Ranvier (Maggi *et al.*, 2003). Nodes of Ranvier contain a high density of sodium channels, which are important for the propagation of an action potential to "jump" from one node of Ranvier to another, known as saltatory conduction (Delfiner, 1996; Mackinnon, 2002). In contrast, in unmyelinated axons an action potential must travel along the entire nerve as a continuous wave. Consequently, action potentials propagate much faster in myelinated nerve fibers (Maggi *et al.*, 2003).

3. Topography of Peripheral Nerves:

Peripheral nerve fibers are coalesced into bundles (known as fascicles) within the nerve trunk (Delfiner, 1996). According to Stewart *et al.* (2003), based on microelectrode recordings on human nerves, nerve fibre types are organized within fascicles. In other words, both myelinated and unmyelinated nerve fibers are separated with respect to their function. However, nerve fibers can cross from one fascicle to another and can split and merge with other fascicles (Delfiner, 1996). Plexus formation (i.e., intermingling of nerve fibers between fascicles) is more extensive in the proximal segments of the nerve than in the distal segments (Sunderland, 1945). Due to the clustering of nerve fibers partial focal injury to a nerve trunk, such as nerve compression at the wrist, may selectively injure only certain fascicles and spare others producing restricted sensory and motor deficits (Stewart, 2000).

4. Connective Tissues:

Connective tissues within the nerve trunk provide some protection to fascicles and individual nerve fibers against nerve compression (Sunderland, 1990; Delfiner, 1996). Connective tissues also provide the nerve fibers with mechanical support, nutritional support,

and well-endowed blood supply (Delfiner, 1996). The connective tissue layers found in the nerve trunk, from the outside to the inside of the nerve trunk, are the following: a) mesoneurium, b) epineurium, c) perineurium, and d) endoneurium (Fig. 1-1).

The mesoneurium is loose connective tissue that comprises the exterior of nerve trunks (Maggi *et al.*, 2003). It allows longitudinal excursion of nerves (known as gliding of a nerve) during movement of an extremity (Maggi *et al.*, 2003). For example, during wrist flexion the median nerve can move up to 9.6 mm, while the median nerve moves slightly less during extension (Millesi *et al.*, 1990). In moderate and severe CTS fibrosis or injury to the mesoneurium can occur and impair or inhibit nerve gliding (Mackinnon, 2002). Injury to the mesoneurium results in the nerve adhering to surrounding tissues. Consequently, further movement causes traction on the nerve, producing further nerve dysfunction and pain or discomfort (Mackinnon, 2002).

The epineurium is a connective tissue layer that is continuous with the mesoneurium (Landers & Altenburger, 2003; Maggi *et al.*, 2003). The epineurium contains the main channels of the intraneural blood supply (Lundborg & Dahlin, 1989). It is composed of two layers: an interfascicular (i.e., internal) and perifascicular (i.e., external) epineurium (Landers & Altenburger, 2003). The internal epineurium is loose connective tissue, comprised of collagen fibrils, which surrounds an individual fascicle (Sunderland, 1990) and helps the fascicles move longitudinally amongst themselves (Landers & Altenburger, 2003). The internal epineurium also cushions and protects the fascicles against external compression by acting as a shock absorber (Sunderland, 1990). In contrast, the external epineurium is a dense layer of connective tissue, comprised mainly of collagen and elastic fibers, which makes up the outer sheath of the nerve (Maggi *et al.*, 2003). The epineurium maintains the structural integrity of the nerve and coalesces the fascicles (Landers & Altenburger, 2003).

The perineurium is a sheath that surrounds each fascicle, protecting them from longitudinal stretch (Landers & Altenburger, 2003). It consists of multilayered perineurial cells

separated by collagen (Delfiner, 1996; Maggi *et al.*, 2003) that are linked together by tight junctions (Delfiner, 1996). The tight junctions are selectively permeable (Maggi *et al.*, 2003), which makes the perineurial layer an integral part of the blood-nerve barrier (Delfiner, 1996) (see section 1-II-7). Briefly, the blood-nerve barrier isolates the fascicles from the surrounding tissues, maintains homeostasis of the endoneurial fluid (Stewart, 2000) and sustains an intrafascicular pressure for fascicles (Sunderland, 1990). The endoneurial fluid is located within the endoneurial space, which is a compartment within the perineurium. In addition to protecting the environment surrounding the nerve fibers, the perineurium provides most of the tensile strength and elasticity to the peripheral nerve (Maggi *et al.*, 2003; Sunderland, 1990). Consequently, the nerve maintains its elasticity if the perineurium is not damaged (Sunderland, 1990).

The endoneurium is relatively loose connective tissue located within the endoneurial space of the perineurium (Landers & Altenburger, 2003). Similar to the perineurium, the endoneurium displays some tensile strength (Maggi *et al.*, 2003; Sunderland, 1990). It is composed of collagen fibrils and fibroblasts, but lacks elastin (Landers & Altenburger, 2003). The more superficial a nerve is, the more collagen fibrils it contains, possibly due to the fact that the nerve requires more protection against nerve damage (Sunderland, 1978). The endoneurium surrounds individual nerves, forming endoneurial tubes around individual axons and associated SCs (Landers & Altenburger, 2003). Therefore, it holds the individual nerve fibers together in the nerve trunk (Dawson *et al.*, 1999; Maggi *et al.*, 2003). The endoneurium also provides resistance to the elongation of the nerve fibers (Landers & Altenburger, 2003).

5. Axoplasmic Transport:

Like the connective tissue layers, axoplasmic transport (also called axonal transport) helps to maintain the integrity of the nerve (Lundborg & Dahlin, 1996). More importantly, it is essential for axonal growth and survival (Lundborg & Dahlin, 1996). Axoplasmic transport is an

energy-dependent process that moves materials away (anterograde) and towards (retrograde) the soma, occurring within the axoplasm (Maggi *et al.*, 2003). Axoplasmic transport does so with the help of motor proteins (dynein and kinesin), which transport substances to and from the soma, respectively, along microtubules (Schnapp & Reese, 1989).

The axoplasmic transport is important because axons extend large distances from the soma and axoplasmic transport provides communication between the soma and different parts of the axon (Maggi *et al.*, 2003; Lundborg & Dahlin, 1996). Elements required for the axon and axon terminals, such as proteins, neurotransmitters, mitochondria, membranous vesicles, lipids, and RNA are synthesized within the soma (Stewart, 2000). These elements are important for the integrity and proper functioning of the distal parts of the axon (Maggi *et al.*, 2003). Therefore, they are transported from the soma by anterograde axoplasmic transport (via kinesins; Stewart, 2000), which transports substances distally along the axon at either a slow or fast rate depending on the substance being carried (Maggi *et al.*, 2003). Slow anterograde axoplasmic transport occurs at a rate of 1 - 6 mm/day and is used to transport structural components including actin, tubulin, microfilaments, and microtubules (Maggi *et al.*, 2003). In contrast, the fast anterograde transport, at a maximal rate of 410 mm/day (Rempel *et al.*, 1999), is used to transport elements that are important functionally at the axon terminals, such as enzymes, neurotransmitters, glycoproteins, and lipids (Maggi *et al.*, 2003; Lundborg & Dahlin, 1996; Rempel *et al.*, 1999).

Besides the anterograde axoplasmic transport in peripheral nerves, there is also a retrograde axonal transport system, which transports substances back to the soma from the axonal terminal via dynein (Maggi *et al.*, 2003). Retrograde axoplasmic transport only involves fast transport, which has a velocity of up to 300 mm/day (Lundborg & Dahlin, 1996). Retrograde axoplasmic transport is mainly used to transport tropic and trophic factors (synthesized by peripheral targets) and dispose of waste material (Lundborg & Dahlin, 1996). At the soma these substances induce necessary metabolic changes. Therefore, retrograde transport is thought to

provide information to the soma about the "status of the axon, its terminals, environment, and target tissues" (Cheng, 2002). Since the axon relies on axoplasmic transport to obtain its proteins and other important substances, disruption of axoplasmic transport, for example due to chronic nerve compression, would cause the axonal segment distal to the injury to degenerate in a process known as Wallerian degeneration (see section 1-IV-1).

6. Blood Supply to the Peripheral Nerve:

The continuous energy supply required to maintain axoplasmic transport and impulse conduction is provided by a well-developed intraneural vascular system (Lundborg, 1979). The intraneural vascular system is located within the layers of the epineurium, perineurium, and the endoneurial space (Lundborg & Dahlin, 1996). The blood supply to the peripheral nerves is critical for the survival and physical integrity of axons (Sunderland, 1990). Compression can interfere with the intraneural microcirculation and can cause sensory deficits, such as paresthesias (Lundborg & Dahlin, 1996).

According to Maggi *et al.* (2003), blood is supplied to the peripheral nerves through extensive connections between the intrinsic and extrinsic vascular systems The extrinsic system arises from small vessels on the surface of the nerve. Small arteries enter the nerve through the mesoneurium at several locations along the nerve trunk where they communicate with the endoneurial space through the vasa nervorum (i.e., the blood vessels supplying the nerves). Surrounding blood vessels in tissues anastomose with vessels that traverse longitudinally within the epineurium, perineurium and the endoneurial space of the perineurium (Delfiner, 1996; Maggi *et al.*, 2003).

Vessels of the perineurium enter the endoneurium at an oblique angle and anastomose with the intrinsic system (endoneurial vessels) that circulates around each nerve fascicle (Maggi *et al.*, 2003). The entry of the perineurial vessels at an oblique angle makes a "valve mechanism," which is susceptible to compression by external or internal forces (Lundborg &

Dahlin, 1996). For example, at this junction circulation may be compromised if there is an increase in endoneurial pressure via intrafascicular edema (Maggi *et al.*, 2003). Ischemia of a nerve produces edema within the endoneurium (Stewart, 2000). The nerve is restricted by the perineurium and the subsequent increase in pressure, due to the ischemia, compromises microcirculation and further impairs the function of the nerve (Stewart, 2000). Focal ischemia is prevented to some extent due to the extensive connections of the main extrinsic nutrient vessels (Delfiner, 1996).

The vasa nervorum is so well organized that peripheral nerves typically have a continuous supply of blood even during mobilization and when local regional vessels are blocked or injured (Sunderland, 1990). The blood vessels are coiled and relaxed, allowing many gliding movements of the nerve trunk to occur without damage to the nerve (Stewart, 2000; Lundborg & Dahlin, 1996). However, when blood vessels are damaged, due to structural changes of the nerves or surrounding tissues, the blood supply can be interrupted or there can be localized interference, resulting in nerve dysfunction (Rydevik *et al.*, 1981).

7. Blood-Nerve Barrier:

Within fascicles, an endoneurial network of capillaries forms a 'blood-nerve barrier', maintaining the proper functioning of axons by keeping a highly controlled milieu within the endoneurial environment (Delfiner, 1996). The blood-nerve barrier is comprised of the internal layers of the perineurium and the endothelial cells of the endoneurial blood vessels, which are linked by tight junctions (Maggi *et al.*, 2003; Stewart, 2000). The tight junctions monitor the intraneural environment and provide a level of immunologic protection to the nerve fibers (Maggi *et al.*, 2003).

Within the cytoplasm of the perineurium are pinocytotic vesicles that are thought to transport material across the blood-nerve barrier (Delfiner, 1996). The barrier is permeable only to simple sugars, unless it is damaged (Landers & Altenburger, 2003). A nerve injury, such as

compression, can impede the blood-nerve barrier at the level of the microvessels (Maggi *et al.*, 2003). Leaking microvessels in the endoneurium results in an accumulation of proteins and the entry of lymphocytes, macrophages, and fibroblasts within the endoneurial space, which used to be in the perineurial space (Delfiner, 1996). Therefore, there is a loss of homeostasis within the endoneurial environment (Mackinnon, 2002) that leads to deleterious changes in the conductive properties, structure of the axons, and myelin (Delfiner, 1996). Due to the lack of lymphatic circulation and unresolved edema within the endoneurial space, the endoneurial fluid pressure increases and produces ischemic damage within the fascicle (Maggi *et al.*, 2003; Mackinnon, 2002).

1 – III Nerve Compression:

Nerve compression can disrupt or stop blood supply and axoplasmic transport. Rydevik *et al.* (1981) examined the effect of graded compression on intraneural microcirculation by applying external pressures to a tibial nerve of a rabbit. It was determined that with 20 mm Hg of external pressure on the nerve there was a decrease in venule blood flow, with 30 mm Hg there was disruption of axonplasmic transport in the nerves, and with 80 mm Hg, intraneural blood flow was stopped completely.

Nerve compression results in the following histopathological changes in a hierarchical order: break-down in the blood-nerve barrier, followed by subperineurial edema, which leads to endoneurial and subperineurial edema, then an increase in thickening of the internal epineurium and perineurium, subsequently localized segmental demyelination occurring at paranodal regions in myelinated nerve fibers (Maggi *et al.*, 2003), followed by diffuse demyelination, and degeneration of nerve fibers distal to the lesion (Wallerian degeneration) eventually occurring in advanced stages (Maggi *et al.*, 2003).

1. Pathophysiology of Nerve Compression:

The severity and extent of nerve injury due to compression depends upon both the magnitude and the duration of the compression (Mackinnon, 2002). The damage to the nerve fibers is more severe the longer the duration and the larger the magnitude of compression (Cheng, 2002). Although, not all nerve fibers are equally affected by compression (Maggi *et al.*, 2003), nerve fibers most susceptible to injury are: relatively large (Dahlin *et al.*, 1989), located superficially within an individual fascicle, (Powell & Myers, 1986) within fascicles that are located peripherally within a nerve trunk, and/or located within large fascicles that are embedded within a small amount of epineurium (Cheng, 2002). Generally, individual nerves are more sensitive to compression if the individual has a generalized peripheral neuropathy or if there is some damage to nerve fibers proximally ("double-crush" hypothesis; Stewart, 2000). The double-crush hypothesis refers to the notion that a partial proximal lesion of nerve fibers disrupts axoplasmic transport, consequently making the distal segment of the nerve more vulnerable to injury (Upton & McComas, 1973). There may also be a reverse double-crush syndrome, referring to a distal nerve injury increasing the susceptibility of an injury more proximal via disruption of axoplasmic transport (Stewart, 2000).

2. Degrees of Nerve Injury:

The degree of nerve damage affects the function of the nerve and its potential for recovery. Nerve fibers are affected by compression to various degrees (Maggi *et al.*, 2003). As aforementioned, the extent of damage to each never fibre depends upon their size and intrafascicular topography (Cheng, 2002). Therefore, nerve compression typically results in a mixed lesion. It remains useful to define the stages of nerve compression based upon either the degree of tissue damage, prognosis, and/or time for recovery (Seddon, 1943). Typically, injuries are categorized either by the Seddon or Sunderland classification systems (Table 1-1). Seddon

separates nerve injuries into three types: neurapraxia, axonotmesis, and neurotmesis. It is based upon the degree of tissue damage, prognosis, and time for recovery (Seddon, 1943). In Sunderland's classification system Seddon's nerve injury types are expanded to five types of nerve injuries: types 1-5. The Sunderland scheme is based upon the extent of trauma to the axon and its supporting connective tissue structures (Sunderland, 1978).

Neurapraxia represents a moderate level of nerve damage and corresponds to Sunderland's type 1 nerve injury (Delfiner, 1996). Axonal continuity is preserved and there is no degeneration of the nerve fibers in the distal nerve stump (Wallerian degeneration). However, a local blockage in the nerve that precludes the transmission of impulses across a segment of nerve (i.e., conduction block) does occur (Dumitru, 1995b). The recovery of conduction over the compressed segment requires weeks or months, corresponding with the time required for the myelin sheath to be repaired and remyelinated (Seror, 2002). Typically with neurapraxia, an individual experiences a mild sensory deficit, paresthesia, and impaired sensory discrimination and interpretation (Dorfman, 1990). Given that the axonal continuity is preserved, muscles innervated by the nerve have minimal changes, and can still be activated distally from the nerve lesion; there is a good chance of full recovery (Robinson, 2000).

Nerve injuries classified as axonotmesis (equivalent to Sunderland's types 2- 4 injuries) indicates a more severe injury than neurapraxia (Dorfman, 1990). Typical etiologies are profound nerve compression or traction. Although the endoneurial tube remains intact, the axonal continuity is disrupted, and Wallerian degeneration occurs (see section I-VI-I; Seror, 2002). The extent of tissue damage varies and the individual may suffer severe paresthesia. Recovery from axonotmesis requires axonal regeneration (Robinson, 2004). Briefly, following degeneration of the distal nerve stump, growing axonal sprouts emerge from the proximal nerve stump and enter endoneurial tubes that act as conduits for the axonal sprouts to grow back and reinnervate the end target (Stoll & Muller, 1999). The growing axonal sprouts may encounter various challenges that could avert the sprouts from reaching the end target. If an axon does not

reinnervate the appropriate end target in a certain period of time, if ever, there are various adverse consequences (Evans, 2000). Such consequences include muscle atrophy, complete loss of muscle function, abnormal sensation, and painful neuropathies (Evans, 2000). The time of sensory and motor recovery depends on the rate of axonal regeneration and reinnervation of denervated motor or sensory end organs often requiring months.

Neurotmesis (corresponding to Sunderland's types 3-5 injuries) is the most severe nerve injury; the nerve is transected (Landers & Altenburger, 2003). Both the endoneurial tubes and the connective tissue of the trunk are damaged, increasing the possibility of the axons being misdirected to inappropriate end targets (Sunderland, 1990).

In the case of CTS, the nerve injury is either categorized as neurapraxia (type 1) or axonotmesis (type 2), depending on the extent of nerve compression. In our study the subjects had moderate or severe CTS with substantial axonal loss and no conduction block. Therefore, the CTS cases examined were axonotmesis (type 2) in nature.

1 – IV Regenerative Response:

Following a peripheral nerve injury that disrupts axonal continuity, such as axonotmesis (type 2), a series of cellular and molecular changes occur to and within the soma and within both the proximal and distal nerve stumps (Fig. 1-2; Chaudhry & Cornblath, 1992; Seckel, 1990). The first responses are the retraction of the proximal and distal nerve stumps, axoplasm leakage, and the collapse of the injured membranes (Terenghi, 1999). The proximal nerve stump swells, most likely due to substances that are still being transported anterogradely and are now accumulating (Fu & Gordon, 1997). In the distal nerve stump, the milieu is altered (see below). This allows the axonal sprouts, arising from the proximal nerve stump, to cross the nerve injury site and have a chance to successfully reinnervate appropriate end organs (Fawcett & Keynes, 1990).

1. Distal Nerve Stump:

Upon injury, the parts of nerve fibers distal to the injury site degenerate and the axonal and myelin-derived debris is removed and recycled (Fawcett & Keynes, 1990). This process is known as Wallerian degeneration (WD). WD can be caused by a traumatic, an inflammatory, or an autoimmune injury to the axon (Stoll *et al.*, 2002). WD creates a permissive environment for the regenerating axons to transverse the injury site from the proximal to the distal nerve stump (Fawcett & Keynes, 1990). Typically, WD initially occurs in the distal portion of injured peripheral nerves in which the axoplasm has been disrupted (Stoll *et al.*, 2002). This is known as secondary degeneration (Frostick *et al.*, 1998). However, primary degeneration can also occur, in a few nodes of Ranvier just proximal to the site of nerve injury (Frostick *et al.*, 1998). Morphological changes are seen within the first 3 days post-injury, beginning as early as 24 hours following injury with the axoplasma and axolemma fragmenting and degenerating (Donoff, 1995). This step is complete within 24 and 48 hours for small and large nerve fibers, respectively (Stoll *et al.*, 1989).

Within 2 days following injury, SCs down-regulate myelin protein synthesis, phagocytize some of the myelin debris, and break down their own myelin sheaths forming ovoids (Stoll *et al.*, 1989). Additional SCs migrate from the proximal and distal nerve stumps. By day 7, macrophages enter the injury site (Perry *et al.*, 1987). The macrophages remove the majority of the debris within 15 - 30 days post-injury (Frostick *et al.*, 1998), thereby removing inhibitory myelin substances, such as myelin-associated glycoprotein (Fu & Gordon, 1997). The endoneurial tubes shrink and can remain in this condition for months, waiting for a regenerating axon to arise proximal from the injury site. However, after approximately a year the endoneurial tube further decreases to about half the size of its original cross-sectional area (Sunderland & Bradley, 1950). Following axon and myelin degeneration, the SCs remaining inside the empty

endoneurial tubes, which originally surrounded the nerve fibre, proliferate in the distal nerve stump (Donoff, 1995; Frostick *et al.*, 1998).

In *vitro* studies, both the axolemma and myelin debris initiate SC mitosis (Salzer & Bunge, 1980). The SCs fill in the space left by the axon and myelin sheath, aligning themselves within the endoneurial tubes in the distal nerve stump (Torigoe *et al.*, 1996). These SCs form a continuous tissue cable across the gap between the proximal and distal nerve stumps, known as the bands of Bungner (Torigoe *et al.*, 1996). The bands of Bungner facilitate a favorable environment for peripheral axonal regeneration (Frostick *et al.*, 1998), acting as a substrate for the emerging axonal sprouts from the proximal nerve stump and expressing surface molecules that help direct the regenerating axons (Stoll & Muller, 1999).

Both macrophages and SCs secrete mitogens and growth factors that help facilitate axonal regeneration and remyelination (Reynolds & Woolf, 1993). There is an up-regulation of cytokines, neurotrophins, neural cell adhesion molecules and their receptors (Stoll & Muller, 1999). When axonal sprouts reach the bands of Bungner, a second phase of SC proliferation occurs (Terenghi, 1999). However, if there is a major delay in axonal regeneration the SCs decrease progressively in number and become less responsive (Terenghi, 1999).

2. Soma:

The soma must survive the neural insult in order for the nerve to continue functioning and for there to be a chance at axonal regeneration. Following peripheral nerve injury it is thought that neurotrophic factors, typically transported retrogradely from the target to the soma, are no longer transported and this initiates anatomical changes, and alterations in both gene expression and cellular metabolism within the surviving somas (Fawcett & Keynes, 1990). There is an up-regulation in the synthesis of neurofilament proteins, in the expression of immediate-early genes and in the synthesis of proteins needed for axonal growth, such as growth-associated proteins (GAPs) (Fawcett & Keynes, 1990; Seckel, 1990). The amount of axonal

reaction (changes within the soma) depends upon the extent and location of the neural damage and if it is motor or sensory cells. The axonal reaction increases with the greater the nerve damage the more proximal the nerve damage is to the soma. The changes within the soma are also greater if the soma is sensory and not motor.

According to Fawcett and Keynes (1990), new genes and proteins are made within the soma, correlating with the onset of regeneration. The proteins produced are the same ones made in embryos needed for axonal growth. The soma increases the synthesis of GAPs, tubulin, and actin. Therefore, substances required for reconstructing a nerve fibre. During regeneration both the synthesis and transport of tubulin is augmented.

Axonal growth is reinitiated by metabolic changes in the soma (Fawcett & Keynes, 1990). The most prominent and consistent morphological changes in the soma are as follows: swelling of the soma, nucleolar enlargement, nuclear eccentricity, and the dispersal of Nissl substance (known as chromatolysis; Seckel, 1990; Fu & Gordon, 1997). The Nissl substance, composed of ribosomes and endoplasmic reticulum, scatters within the soma due to the breakdown of rough endoplasmic reticulum (Fig. 1-3; Fawcett & Keynes, 1990). Chromatolysis reflects a metabolic shift from the production of neurotransmitters for synaptic activity to the production of structural components required for axonal repair and growth, such as actin and tubulin (Seckel, 1990; Fu & Gordon, 1997). These cytoskeletal components are transported via slow anterograde transport along the axon, which corresponds with the maximum rate of axonal regeneration (Seckel, 1990).

Chromatolysis occurs earlier and is more severe the more proximal an injury is to the soma (Fu & Gordon, 1997). This implies that the extent of chromatolysis may be associated with the amount of axonal outgrowth required for reinnervation of end targets (Fu & Gordon, 1997). However, chromatolysis does not appear to be essential for successful axonal regeneration, for DRG neurons regenerate successfully with little chromatolysis (Fu & Gordon, 1997).

3. Axonal Regeneration:

Within a few hours post-injury, axonal sprouts emerge from the node of Ranvier proximal to the injury site (Seckel, 1990). Many sprouts may arise from each axon (Fawcett & Keynes, 1990), potentially maximizing the chances for a sprout to reach its end target (Terenghi, 1999). Some sprouts will "die back" due to axonal pruning caused by a deficiency in a survival signal derived from the target end organ (Brushart, 1993). The remaining axon tips swell, attributed to the presence of smooth endoplasmic reticulum, mitochondria, and microtubules and form growth cones (Seckel, 1990; Fawcett & Keynes, 1990).

A growth cone is a "specialized motile exploring apparatus" (Seckel, 1990). It attempts to go through the distal nerve stump and reinnervate the appropriate target organ (Stoll & Muller, 1999). The growth cone facilitates axonal elongation and guidance of a regenerative axonal sprout (Seckel, 1990). A growth cone makes several extensions known as filopodia (neurites in vitro). The growth cone elongates the regenerating axon via ameboid-like movement of the filopodia. Typically a growth cone is in contact simultaneously with SC basal lamina on one side and SC membrane on the other (Fawcett & Keynes, 1990). The growth cone responds to both contact guidance clues and neurotropic clues provided by the components of the SC basal lamina (i.e. laminin and possibly fibronection) and neurotrophic factors in the periphery or target organs, respectively (Seckel, 1990).

Since the endoneurial tube is preserved when a nerve is compressed, the growth cone is more likely to lead to the appropriate end organ (i.e., less misdirection) than when the nerve is axotomized. Regenerating axons grow in diameter, depending on the size of the parent axon, but do not reach full size until reinnervation with an end target (Fu & Gordon, 1997). Once an axonal sprout reinnervates a target all the other axonal sprouts are withdrawn progressively in a process that may take months or years (Fu & Gordon, 1997). Surface molecules are down-regulated (Stoll & Muller, 1999) and the SCs remyelinate the axon only if the parent axon was

myelinated and upon reinnervation with the end target (Fu & Gordon, 1997). The extent of myelination depends upon the size of the regenerating axon (Simpson & Young, 1945). Synaptic terminals are reestablished.

4. Rate of Axonal Regeneration:

Although WD occurs for both compressed and transected nerves there is a marked difference in the success rate between these injuries. Compressed (crushed) axons are more apt for complete recovery than that of transected axons (Lundborg, 1988). Recovery is often faster for crush injuries (3-4 mm/day) than it is for transected injuries (2-3 mm/day; Bontioti *et al.*, 2003; Lundborg, 1988). The reason for the differences is that in crush injuries the endoneurial tube is preserved, which provides a conduit for the regenerating nerve to travel from the proximal to the distal nerve stump (Stoll & Muller, 1999). In contrast, transection injuries involve disruption of the endoneurial tube that results in a lack of continuity between the proximal and distal nerve stumps (Horch, 1979). In both types of injuries axonal sprouts may enter inappropriate endoneurial tubes and be misdirected to the wrong end target (Fawcett & Keynes, 1990). However, misdirection occurs less often following crush injuries than transection injuries, as the endoneurial tube in which the regenerating axon grows through determines the end target (Fawcett & Keynes, 1990).

Regenerating motor axons preferentially select motor branches even if there is misalignment or a gap of the proximal and distal nerve stumps and given equal access to both motor and cutaneous branches (Brushart, 1988). This is known as preferential motor reinnervation (PMR). At the start of axonal regeneration (2 and 3 weeks) motor neurons project an equal number of axons appropriately to motor pathways and inappropriately to cutaneous pathways, with most projecting to both. Later during regeneration (8 and 12 weeks) the incorrect axons are withdrawn or pruned and most of the axons project correctly to muscle (Brushart, 1988; Brushart, 1993). This process of PMR causes a protracted delay in axonal outgrowth and

axonal regeneration, which decreases the chance for functional recovery (Al Majed *et al.*, 2000). PMR is associated with a phenomenon known as staggered regeneration. Staggered regeneration refers to the protracted period of axonal outgrowth at the repair site (Al Majed *et al.*, 2000). Axons regenerate and/or traverse the repair site at different rates (Al Majed *et al.*, 2000). Although the fastest axons regenerate at a rate of 1-3 mm/day (Gutmann *et al.*, 1942) there are many axons that do not transverse the injury site into the distal nerve stump for days or weeks (Al Majed *et al.*, 2000). Therefore, the widely reported rate of axonal regeneration of 3 mm/day describes only the fastest growing axons (Al Majed *et al.*, 2000). The staggered regeneration was associated with PMR, with the motor axons progressively preferential reinnervating motor pathways (Al Majed *et al.*, 2000). The protracted period of time for axons to cross the repair site increases the time for axonal regeneration, decreasing the chance for functional recovery.

1 - V Nerve Compression With in the Carpal Tunnel:

CTS is the most common peripheral nerve injury (Werner & Andary, 2002). It occurs as a result of the median nerve being compressed underneath the transverse carpal ligament (TCL) as it travels through the carpal tunnel at the wrist (De Krom *et al.*, 1992). Symptoms of CTS include numbness in the hand and pain in the wrist. The median nerve is the only nerve that goes through the carpal tunnel. Therefore, it is the only nerve that is susceptible to compression at this location.

1. Median Nerve:

The median nerve arises in the axilla from the union of parts of the medial and lateral cords of the brachial plexus (Stewart, 2000; McNamara, 2003). It is comprised of the C6 to T1 nerve roots (Hennessey & Kuhlman, 1997) and occasionally the C5 fibers (McNamara, 2003). In the upper arm, the median nerve innervates no muscles. The median nerve goes between the

two heads of the pronator teres (Fig. 1-4; Hennessey & Kuhlman, 1997). In the pronator teres the anterior interosseus branch arises from a branch of the median nerve (McNamara, 2003). The anterior interosseous nerve supplies the radial aspect of the flexor pollicis longus, flexor digitorum profundus, and pronator quadratus (Hennessey & Kuhlman, 1997). In the forearm the median nerve then courses between the flexor digitorum profundus and the flexor digitorum superficialis (McNamara, 2003). Proximal to the wrist the median nerve becomes superficial and lies between the flexor digitorum superficialis and the flexor carpi ulnaris muscles where the palmaris cutaneous branch arises (McNamara, 2003). The median nerve then passes through the carpal tunnel between the flexor carpi radialis tendon and the palmaris longus tendon (if present) (Delaunay & Chelly, 2001; Stewart, 2000).

As the median nerve emerges from beneath the flexor digitorum superficialis muscle (approximately 5 cm proximal to the TCL) it becomes more superficial and is ensheathed by loose connective tissue (Rotman & Donovan, 2002). The loose connective tissue allows the median nerve to move around when the hand shifts position (Rotman & Donovan, 2002). In the hand the median nerve gives off a muscular branch and palmar digital branches (McNamara, 2003). The median nerve provides most of the autonomic nerve fibers to the hand (Jordan & Greider, Jr., 1987). The median nerve innervates the following: the palmar cutaneous branch, the recurrent thenar motor branch, the digital nerves, and the lumbrical muscles (Hennessey & Kuhlman, 1997). The palmar cutaneous branch provides sensation to the radial half part of palm (Hennessey & Kuhlman, 1997; Roman *et al.*, 1987; McNamara, 2003; Roman *et al.*, 1987). However, the palmar cutaneous branch leaves the main branch of the median nerve proximal to the TCL. Therefore, it is spared from compression in the carpal tunnel (Roman *et al.*, 1987). The recurrent motor branch innervates the thenar eminence: opponens pollicis, flexor pollicis brevis and abductor pollicis brevis (Walters *et al.*, 2001). The palmar digital branches provide sensation to the lateral two lumbricals and to the lateral aspect of the thumb, index finger, long

finger and radial half of the ring finger (McNamara, 2003; Hennessey & Kuhlman, 1997). This is known as the median-nerve distribution (Fig. 1-5).

There are some variants in muscular innervation in the upper limb related to the median nerve, such as the Martin-Gruber anastomosis and the Riche-Cannieu anomaly. The Martin-Gruber anastomosis, having an incidence of 7.7-34% (Iyer & Fenichel, 1976), is a nerve anomaly in the forearm with respect to the communication between median and ulnar nerves (Dumitru, 1995a). Typically, the anomaly is a branch from the anterior interosseous nerve to the ulnar nerve (Srinivasan & Rhodes, 1981). The Riche-Cannieu anomaly refers to the abnormal communication of the median and ulnar nerves in the hand. This occurs between the deep branch of the ulnar nerve and the recurrent branch of the median nerve (Dumitru, 1995b).

2. Anatomy of the Carpal Tunnel:

The median nerve and nine tendons (four flexor digitorum profundus tendons, four flexor digitorum superficialis tendons, and flexor pollicis longus tendon) pass through the carpal tunnel (Wilson & Sevier, 2003), with the median nerve being the most superficial structure within the tunnel (Rotman & Donovan, 2002).

Anatomical constraints within the wrist can result in median nerve compression. The carpal tunnel is a small fibro-osseous canal in the wrist (Cobb *et al.*, 1993). At the proximal end, the mean width of the carpal tunnel is approximately 25 mm (Cobb *et al.*, 1993). However, the cross-sectional area of the carpal tunnel becomes narrower 2.0 – 2.5 cm distal to the entrance (Robbins, 1963), with a cross-sectional area of 20 mm at the level of the hamate hook (a wrist bone; Fig. 1-6; Cobb *et al.*, 1993). The carpal tunnel is confined on three sides by carpal bones and roofed by a thick ligament, known as the transverse carpal ligament (TCL; Robbins, 1963; Fernandez *et al.*, 1997). The TCL is fused to the following wrist bones: 1) the pisiform; and 2) the hook of hamate, ulnarly; and 3) the scaphoid tubercle, 4) the crest of trapezium, and sometimes, 5) the styloid process, radially (Grokoest & Demartini, 1954). The TCL has a

fusiform shape, being thin at its proximal end (0.6 – 2.0 mm), thick in the middle (1.6 - 3.6 mm), and thin at its distal end (0.6 – 1.0 mm; Tanzer, 1959).

Median nerve compression can be at the level of the proximal edge of the TCL, most likely due to the transition between fascia and TCL (Cobb *et al.*, 1993), and/or at the level of the hook of hamate (Robbins, 1963; Phalen, 1966). Compression at the hook of hamate (the narrowest part of the carpal tunnel) is most likely not caused by wrist position, but attributed to an increase in intracarpal contents (Cobb *et al.*, 1993).

3. Etiology of Carpal Tunnel Syndrome:

The etiology of CTS remains equivocal (Franzblau & Werner, 1999), with most cases being idiopathic (Marshall, 2003). Due to the crowding of the structures in the carpal tunnel, the compression of the median nerve within the carpal tunnel may be attributed to the following: a) a thickening of the TCL, b) anomalous structures within the carpal tunnel, c) a slight increase in carpal tunnel contents, or d) a slight decrease in the cross-sectional area of the carpal tunnel (Robbins, 1963; Gelberman *et al.*, 1981).

A reduction in the volume of the carpal tunnel can increase the pressure within the carpal tunnel (known as intracarpal canal pressure; Robbins, 1963). Pressure can be increased by reducing the volume of the carpal tunnel via flexion or extension of the wrist, isolated finger flexion, power fist, or holding objects (Robbins, 1963). A study was conducted on 15 subjects with CTS and 12 healthy controls using a wick catheter to investigate intracarpal canal pressures (Gelberman *et al.*, 1981). The intracarpal tunnel pressure was determined to be lowest when the wrist was in a neutral position (Gelberman *et al.*, 1981). The intracarpal canal pressure was found to be much higher in subjects with CTS (32 mm Hg) compared to healthy controls (2.5 mm Hg) when the wrist was in a neutral position. During wrist flexion of 90 degrees, the pressure rose to 94 mm Hg and 31 mm Hg for the CTS group and controls, respectively. The mechanism by which the resting carpal pressure increases in individuals with

CTS remains uncertain (Burke *et al.*, 2003). One possibility for the rise in resting carpal pressure is the thickening of the synovial lining of tendons (i.e., inflammation; Werner & Andary, 2002). This could increase the volume of the contents within the carpal tunnel, subsequently compressing the median nerve against the TCL, and causing CTS (Werner & Andary, 2002).

Highly repetitive manual activity may cause the synovial lining of tendons to thicken, thereby increasing the contents of the carpal tunnel, subsequently increasing the intracarpal canal pressure and compressing the median nerve (Werner & Andary, 2002). Repetitive manual work (Silverstein *et al.*, 1987) and wrist vibration (Bovenzi *et al.*, 1991) are positively associated with the development of CTS. Consistent with this is a higher prevalence of CTS in occupations requiring intensive manual labor (Silverstein *et al.*, 1987).

Another possible cause for an increase in intracarpal pressure, although rare, is being genetically prone to CTS, such as having a small carpal tunnel, anomalous muscles, or a persistent median artery (Gorkhaly, 2003). A variety of systemic conditions have also been associated with CTS, such as rheumatoid arthritis, diabetes mellitus, hypothyroidism and alcoholism (Maggi *et al.*, 2003). A systemic condition can result in a generalized peripheral neuropathy, which increases the susceptibility of nerves to compression (Stewart, 2000). Some other risk factors for developing CTS are kidney dialysis (Radecki, 1997), acromegaly (overproduction of hormones; Aroori & Spence, 2008), gout (Champion, 1969), pregnancy (Stevens *et al.*, 1992), and menopause (Radecki, 1997). Obesity, age and gender affect the susceptibility to CTS, but the exact associations are unknown (Becker *et al.*, 2002). Women are more prone than men to CTS. Generally, 0.6% of men and 5.8% of women have CTS (De Krom *et al.*, 1992). Women become even more susceptible as they age by a factor of 4 to 1 (Atroshi *et al.*, 1999). Typically, CTS affects individuals over 30 years old (Stevens *et al.*, 1988), with a peak incidence of CTS in the late 50s (Bland & Rudolfer, 2003).

4. Symptoms and Signs of Carpal Tunnel Syndrome:

Cardinal symptoms of CTS are nocturnal pain and numbness, which often awakens the individual, and pain and numbness that is precipitated or aggravated by manual activity that can be relieved by shaking the hand (Hennessey & Kuhlman, 1997). A clinical history of numbness, paresthesia, pain, and weakness in the median nerve distribution can make CTS suspect (Hennessey & Kuhlman, 1997; Patterson & Simmons, 2002). CTS constitutes a constellation of symptoms and signs (Goodyear-Smith & Arroll, 2004). Generally at the earliest stages of CTS there is an absence of sensory findings. However, as the CTS gets worse, sensory findings vary from slight hyperesthesia in the median nerve distribution of the hand to extreme loss of sensation (Dawson *et al.*, 1999). Frequently, individuals with CTS cannot discern where the sensory disturbance is located, due to the dysfunction of the autonomic nerve fibers of the median nerve compressed in the carpal tunnel, and in turn localize it to the entire hand (Hennessey & Kuhlman, 1997). Other rare CTS symptoms, such as dry skin, color changes, or swollen hands may also occur (Phalen, 1966).

According to Hennessey and Kulman (1997), the thumb or index finger also becomes weaker, as CTS progresses, due to thenar muscle atrophy. Individuals with CTS may complain of difficulty holding items or dropping them due to motor and/or sensory disturbances. Occasionally, some individuals complain of pain in their shoulder, elbow, or forearm. This is possibly attributed to CTS, for the median nerve has nerve fibers arising from the C6-T1 and sometimes the C5 nerve fiber root levels.

Early on in the progression of CTS, CTS symptoms are intermittent in nature (Burke *et al.*, 2003). Therefore, the symptoms may be absent when the individual goes to the clinician. Provocative tests are then used to try to elicit the CTS symptoms. The most common provocative tests are: the Hoffman-Tinel sign and the modified Phalen sign. A positive Hoffman-Tinel sign occurs when tingling is felt within the median-nerve distribution following percussion of the compressed median nerve, more specifically at the tip of regenerating sensory axons

(Hennessey & Kuhlman, 1997). The sign is only present if there is axonal loss of sensory nerve fibers (Kipp & Wilson, 2001). Therefore, a positive test carries more weight for clinical diagnosis of CTS than a negative test (Kipp & Wilson, 2001). The modified Phalen sign is a test used to precipitate or aggravate the tingling in the median nerve distribution (Hennessey & Kuhlman, 1997). An individual is asked to press the dorsum of their hands together with the wrists in complete flexion for 30 s – 1 min (Hennessey & Kuhlman, 1997). This test is negative in advanced sensory loss (Wilson & Sumner, 1995). Some studies have suggested both provocative tests have low sensitivity and specificity (Wilson & Sumner, 1995). Indeed, there is a large proportion (i.e., over 20%) of healthy individuals with a positive Hoffman-Tinel sign and modified Phalen sign (Seror, 1988; Seror, 1987). The lack of sensitivity may be attributed to the difference in how the nerve responds to injury, which is dependent on the severity of the nerve compression (MacDermid, 1991).

5. Clinical Correlation to Nerve Compression:

CTS is a classic example of chronic nerve compression (Werner & Andary, 2002; Lundborg & Dahlin, 1996). Various clinical stages of nerve compression correlate with changes in the: a) intraneural microcirculation, b) nerve fiber structure, c) vascular permeability, and e) nerve function (Lundborg & Dahlin, 1996). Sensory complaints progress from intermittent paresthesia to constant numbness, while motor complaints progress from pain to weakness to muscle atrophy (Mackinnon, 2002). In the early stages of CTS, intermittent paresthesia is probably due to disturbances in the intraneural microcirculation (caused by edema within the endoneurial space) and inflammation (Donoff, 1995; Rempel *et al.*, 1999). Changing hand position or shaking the hand relieves the sensory disturbances, resuming circulation. Initially, the CTS symptoms occur only at night (Lundborg & Dahlin, 1996). During sleep the median nerve may become ischemic and generates ectopic impulses that are perceived as paresthesias in the fingers innervated by the median nerve (Lundborg & Dahlin, 1996). Ischemia

at night may be due to the following: a) wrist flexion, b) decrease in systemic blood pressure and blood pressure in intraneural vessels, c) no active muscle pump in the arm; and/or d) redistribution of tissue fluid in the arm (Lundborg & Dahlin, 1996).

As CTS progresses the symptoms become even worse and constant, occurring during both day and night (Lundborg & Dahlin, 1996; Rempel *et al.*, 1999). A mixed nerve lesion occurs. In other words, some nerve fibers are affected by metabolic disturbances due to edema and others are affected by local changes in the myelin sheath (i.e., neurapraxia or axonotmesis) (Lundborg & Dahlin, 1996). Typically, weakness or atrophy of the median-innervated thenar muscles leads to impairment in both sensibility and manual dexterity (Lundborg & Dahlin, 1996). With persistent nerve compression, morphological changes may occur, such as segmental demyelination due to fibrosis (Rempel *et al.*, 1999). At this stage carpal tunnel decompression surgery relieves the individual of symptoms attributed to metabolic disturbances. However, in cases with axonotmesis, demyelination is diffuse and there is disruption in axonal continuity, which causes WD (Maggi *et al.*, 2003; Mackinnon, 2002; Donoff, 1995). Therefore, following surgery the axons still have to regenerate and reinnervate the appropriate end target in an attempt to restore functionality (Lundborg & Dahlin, 1996).

I – VI. Electrodiagnosis of Carpal Tunnel Syndrome:

Currently there is no gold standard for diagnosing CTS (Manktelow *et al.*, 2004; Rempel *et al.*, 1998) for debate continues over whether diagnosis should be based upon clinical symptoms and signs and/or electrophysiological studies (Atroshi *et al.*, 1999). Therefore, CTS is often diagnosed based upon both (Rempel *et al.*, 1998).

Two electrodiagnostic techniques (nerve conduction studies and needle EMG) are helpful in diagnosing CTS. The nerve conduction (NC) study involves stimulating the nerve with ES and examining the integrity, speed, and time of the electrical current (action potentials) through the nerve (Mallik & Weir, 2005). The presence of a nerve injury is detected by

noticeable slowing or blocking of nerve conduction. In CTS the maximal slowing of the conduction velocity is across the wrist (Casey & Le Quesne, 1972). The needle EMG involves inserting a fine needle into muscles of interest. Muscles are recorded at rest and during contraction. Needle EMG records the electrical activity of the muscles and helps to determine the extent of nerve injury (Mills, 2005).

1. Median Nerve Conduction Studies:

Simpson was the first to demonstrate the usefulness of median motor NC studies in the diagnosis of CTS (Simpson, 1956). Median motor NC studies are helpful in evaluating the involvement of motor nerves, the function of the thenar muscle, and the localization of the compression (Stevens, 1987). In motor NC studies the terminal motor latency and the compound muscle action potential (CMAP) amplitude are determined. The characteristic abnormalities found in CTS patients on NC studies are focal slowing at the carpal tunnel and decreased sensory nerve action potential (SNAP) and CMAP amplitudes (Kiernan *et al.*, 1999).

The terminal motor latency (also known as the distal latency) is the interval between the onset of stimulation and the onset of the CMAP (described below; Dumitru, 1995b). In advanced CTS the median terminal motor latencies are prolonged across the carpal tunnel (> 4.2 ms), but typically are normal distal and proximal to the TCL (Stevens, 1987). Typically to evaluate if the median terminal motor latency is abnormal in the symptomatic hand a comparison is made with the other hand. However, because in 58% of CTS cases CTS occurs bilaterally (Stevens, 1997) the parameters in our study will be compared with standard normative values (Table 1-2; Dumitru, 1995b).

The CMAP (also known as the M response) is the summated electrical activity of all the depolarized motor fibers innervated by a depolarized nerve (Dumitru, 1995b). The CMAP may be reduced in size (< 4 mV) or may be absent due to axonal loss (Stevens, 1987). In slowly

progressive compression injuries the CMAP amplitude may continue to decrease over weeks or months (Dumitru, 1995b). However, several weeks following partial lesions intact nerves may extend collateral axonal sprouts to reinnervate deneravated motor fibers (known as collateral sprouting). The collateral sprouting limits the CMAP amplitude as a good indicator of axonal loss because there is no longer a 1-to-1 relationship between the number of axons lost and the size of the CMAP (Dumitru, 1995b).

Although both sensory and motor axons may be affected by CTS the sensory NC studies are generally thought to be more sensitive at detecting early abnormalities than motor NC studies (Cioni *et al.*, 1989). The SNAP and transcarpal CV are recorded. The SNAP is the summated activity of electrical activity generated by sensory nerve fibers in response to ES (Dumitru, 1995b). In contrast to the CMAP that records the response of a muscle, the SNAP is recorded from the nerve itself by surface electrodes. The transcarpal CV refers to the speed of the propagation of an action potential across the carpal tunnel (Dumitru, 1995b). Focal slowing of the CV across the wrist occurs in cases of CTS.

The presence of prolonged latencies across the carpal tunnel, but preserved amplitudes (i.e., SNAP and CMAP) indicates local demyelination (focal damage to the myelin sheath) (Ochoa & Marotte, 1973; Kiernan *et al.*, 1999; Stevens, 1997). Conduction block or temporal dispersion of the compound volley, or axonal loss of sensory nerve fibers and motor nerve fibers is demonstrated by a reduction in the size of the SNAP and maximal CMAP (Kiernan *et al.*, 1999; Dawson *et al.*, 1999). The reduction in SNAP is associated with sensory loss and the decrease in CMAP is associated with thenar muscle weakness (Stevens, 1997).

To exclude the possibility of another peripheral neuropathy (i.e., disease or dysfunction of one or many peripheral nerves) causing the prolonged median nerve latencies, NC studies are conducted on the ulnar and radial nerves and occasionally needle EMG is performed (Stevens, 1997). Needle EMG helps define if the compression is due to CTS or another condition, such as a peripheral neuropathy, proximal median nerve compression, C-6, C-7, or

C-8 radiculopathies (i.e., disease of the spinal roots; Stevens, 1997). It also helps to determine the extent of nerve injury by the presence of fibrillations and abnormalities of motor unit potentials (Stevens, 1997). Muscles examined when NC studies suggest CTS may include: a) abductor pollicis brevis; b) flexor pollicis brevis; c) first dorsal interosseous; d) pronator teres; e) flexor carpi radialis muscles; f) biceps; and g) triceps (Stevens, 1987; Stevens, 1997). Needle EMG is performed by inserting a needle electrode into the various muscles innervated by the nerve of interest. The spontaneous and voluntary electrical activity of the muscles is examined (Mills, 2005). The EMG detects the extent of injury of the muscles and associated irritability. An indication of severe nerve compression and axonal motor loss are fibrillation potentials, decreased recruitment of motor units and motor units that have increased in size on needle EMG studies (Stevens, 1997).

A caveat to using only electrodiagnostic techniques to diagnose CTS is that these tests are neither 100% sensitive, nor 100% accurate (MacDermid, 1991). The electrodiagnostic tests poorly correlate with CTS symptoms (Dellon, 1993; Grundberg, 1983). NC studies evaluate the conduction of large myelinated fibers, although most nerve fibers in peripheral nerves have small diameters (A-delta or small myelinated fibers and C or unmyelinated fibers; Thonnard *et al.,* 1999). Therefore, unaffected axons could provide normal CV, while the more severely affected axons cause CTS symptoms. It is known that false-positives occur on the NC studies for the following reasons: a) individuals have Martin-Gruber anastomosis, occurring in 7.7 - 34% of healthy individuals (Iyer & Fenichel, 1976; Heller *et al.*, 1986), b) 1/3 of healthy individuals have false-positive results (Redmond & Rivner, 1988), and c) individuals with normal electrodiagnostic tests have significant symptomatic relief following carpal tunnel decompression surgery (Grundberg, 1983). In our study electrophysiologic data and CTS symptoms and signs were used to diagnose CTS.

2. Motor Unit Number Estimation (MUNE):

To estimate the severity of motor axonal loss, measures more direct than conventional motor conduction studies are needed. A major reason being that the maximal CMAP is not a reliable indicator of motor axon loss both in acute and long standing injuries. In the former case, action potentials associated with newly regenerated motor units are small, polyphasic, thus contributing little to the CMAP. In the latter case when action potentials associated with chronically regenerated motor units are markedly enlarged and can increase the size of the CMAP greatly. Therefore, the CMAP size is often a poor indicator of motor axonal degeneration and subsequent regeneration because it can be too easily confounded by motor branch sprouting and motor unit remodeling. For that reason, more direct methods have been introduced to circumvent these confounding factors.

Motor unit number estimation (MUNE) is a relatively direct electrophysiological technique that can be used to measure change in the relative number of functional motor axons within a muscle or groups of muscle over time. A motor unit is defined as the alpha motor neuron in the spinal cord, its axon, and the muscle fibers the axon innervates. MUNE was introduced by McComas (McComas *et al.*, 1971) to circumvent the problem of not being able to count all the motor units within a muscle in humans. MUNE is an estimate because the true anatomic number of motor units within a muscle is unknown (Felice, 1995). Since the introduction of MUNE, specifically the manual incremental method, various other MUNE techniques have been developed to try to make the estimates more practical and reliable.

For all MUNE techniques, to estimate the number of motor units, the size of the maximal CMAP and the size of the average surface detected motor unit action potential (SMUP) must be determined. The CMAP and SMUPs are measured in either areas or amplitudes of their associated waveforms. The CMAP, also known as the M wave, is obtained by supramaximally stimulating the motor nerve, which subsequently depolarizes all of the motor units of a muscle. The summated response is "not completely synchronous because the constituent motor axons

do not conduct action potentials at the same velocity" (Slawnych *et al.*, 1997). A small, but representative sample of SMUPs within a muscle must also be obtained. A SMUP (surface motor unit action potential) is the unitary signature that corresponds to the activation of a single motor unit. To ensure the potential is not due to the activation of more than one motor axon, a SMUP must meet all the following requirements: it must be evoked with distinct thresholds; reproducible in an all-or-none fashion; and does not fractionate on successive identical stimuli. From this sample an average SMUP is derived. Finally, to estimate the number of motor units within a muscle or muscle group the following equation is used:

$$\text{MUNE} = \frac{\text{size of the maximal compound muscle action potential}}{\text{size of the average surface detected motor unit action potential}}$$

The principles behind the MUNE techniques are similar. All have the same number of underlying assumptions: 1) each SMUP must represent the activity of a single motor unit; 2) the sample of SMUPs must be representative of the different sizes of SMUPs that make up the maximal CMAP; 3) the average SMUP derived from the sample of SMUPs must accurately represent the SMUPs that contribute to the maximal CMAP (Doherty & Brown, 1993). However, how the representative sample of motor units is obtained differs between MUNE techniques.

One of the most commonly used MUNE techniques is the Multiple Point Stimulation (MPS) method. It is a non-invasive technique, introduced by Kadrie *et al.*, (1976). The MPS technique, like all the MUNE techniques, assumes that the sample of motor units is representative of the entire motor unit population (Slawnych *et al.*, 1997). MPS also assumes that stimulating the nerve at threshold only stimulates a single motor axon and there is no systematic bias in the size of SMUPs attained by using threshold ES (Doherty *et al.*, 1995).

To perform MPS, the stimulating electrodes are moved along the superficial course of a peripheral nerve. For example, in the case of the median nerve, it can be stimulated from the distal wrist crease to the distal forearm and then from the elbow to the axilla. Stimulation of the

median nerve over the mid-forearm is too deep to stimulate without discomfort to the subject. At each site along the nerve, the lowest threshold motor axon is activated. Different axons are activated at different points along the nerve. On occasion a second successively higher threshold motor axon at a single site of stimulation can be attained by using software that allows template subtraction. It is done by storing the "null" template that can be subtracted from the next "all" response. The stimulus intensity is carefully increased at fine increments.

There are many advantages of using MPS compared to the other MUNE techniques. First, MPS is relatively noninvasive, requiring only submaximal stimulation to obtain the sample of SMUPs. Indeed, many subjects can sleep through the test sessions. Second, alternation is not a problem. Third, the average SMUP size is based on the actual SMUPs not "an estimate determined by using algorithms that correct for alternation or a statistical estimate of SMUP size" (Doherty *et al.*, 1995).

Like most other techniques, probably the most severe challenge in MPS is to collect a sufficiently large motor unit sample. Because of closely overlapping excitability of neighboring motor axons, this is an often difficult and time-consuming task. In an attempt to overcome this problem, we evaluate the possibility that by administering stimuli with long pulse width, additional motor units can be activated. This idea is based on a number of theoretical studies. By modeling the cable properties of motor axon, Grill Jr. & Mortimer (1996) found that different motor units could be selectively recruited by altering the stimulus pulse width. This is because myelinated nerve fibers of different sizes have different thresholds to stimulus pulses with different pulse width. However, in that model, it was assumed that axons were situated in a homogenous medium and the stimulus point source was located immediately adjacent to the nerve. Whether those assumptions are applicable to surface stimulation where there are interposed skin, subcutaneous tissues, tendons and blood vessels between the stimulator and the nerve is unknown. In this thesis, we test this through experiments using surface stimulus in healthy subjects. The results of those experiments are described in chapters 2 and 3.

3. Treatment for Carpal Tunnel Syndrome:

Once CTS is diagnosed it should be treated early to prevent irreversible median nerve damage, resulting in persistent symptoms and permanent disability (Dawson *et al.*, 1999). The type of treatment used to alleviate the CTS symptoms depends upon the severity of the median nerve compression. When CTS occurs secondarily to a reversible disorder, such as hypothyrodism, or pregnancy, CTS symptoms may be controlled by conservative methods (Hudson *et al.*, 1997). Conservative methods include: the application of anti-inflammatory medications, or wearing a wrist splint (Burke *et al.*, 2003). The wrist splint holds the wrist in a neutral position (Burke *et al.*, 2003). However, a splint is often ineffective in the long-term and the individual may experience continuous paresthesia or numbness (Burke *et al.*, 2003). According to Goodyear-Smith and Arroll (2004), the best conservative treatment for CTS is a steroid injection. In the short-term steroids are effective, especially if the symptoms are mild and intermittent (Babu & Britton, 1994). However, even with conservative treatments, over time the recurrence rate of CTS is high (60% of cases; Phalen, 1972).

Conservative treatments may be inadequate to eliminate the CTS symptoms and decompression surgery is warranted to alleviate the pressure on the median nerve (Burke *et al.*, 2003). The surgery, which involves the transection of the TCL, alleviates the pressure on the median nerve by increasing the carpal tunnel volume by 15 - 20% (Burke *et al.*, 2003). Typically, surgery only takes 10 - 25 minutes and is performed under local anesthesia (Hudson *et al.*, 1997). The risk for decompression surgery is minimal (Agee *et al.*, 1992) and has a high rate of success (Kline, 1990), being effective for all ages (Wilgis *et al.*, 2006). According to Fernandez *et al.* (1997), approximately 90% of patients are relieved of CTS symptoms following surgery. However, Manktelow *et al.* (2004) state that many of the individuals have residual symptoms of CTS 4 years ensuing any CTS treatment. Even with CTS decompression surgery if there is substantial axonal degeneration, axonal regeneration, functional recovery and outcome are

often incomplete. In our study subjects with substantial axonal loss due to CTS will be used to assess the effectiveness of ES on axonal regeneration and functional recovery and outcome.

3. Electrical Stimulation:

Experimental strategies are being explored with hopes of enhancing axonal regeneration and functional outcome ensuing peripheral nerve injury, such as median nerve compression. Three main approaches have been examined: a) manipulation of the SCs; b) application of neurotrophic factors; (Boyd & Gordon, 2003) and c) application of electric fields (Frostick *et al.*, 1998). However, thus far no such treatments are clinically applicable or available. An example of an anticipated clinical treatment for improving axonal regeneration and functional outcome is the application of ES to peripheral nerves. ES has been shown to enhance peripheral axonal regeneration in studies on rats (Pockett & Gavin, 1985; Roman *et al.*, 1987; Al Majed *et al.*, 2000; Mendonca *et al.*, 2003; Pomeranz *et al.*, 1984; Politis *et al.*, 1988), mice (Ahlborn *et al.*, 2007; Geremia *et al.*, 2007), rabbits (Nix & Hopf, 1983), canines (Rozman *et al.*, 2000), and humans (Amirjani, 2005).

For a long time electricity has been known to facilitate healing in different biological processes, such as bones, tendons, and muscles (Mendonca *et al.*, 2003). In the mid-eighteenth century ES was a popular treatment to heal disturbances of the nervous system (Mears *et al.*, 2003). However, ES was forgotten as a potential treatment for some time, owing to suspicion that it was not effective (Brushart *et al.*, 2002). Due to the promising effects of ES, the use of ES as a clinical treatment is being revisited. Various methods of ES, such as weak direct currents and pulsed electromagnetic fields have been applied to peripheral nerves to examine whether electricity can enhance axonal regeneration and improve functional outcome following peripheral nerve injuries (Brushart *et al.*, 2002).

Positive effects of exogenous application of ES have been demonstrated *in vitro* studies. Application of an electrical field to neurites (axons in cell culture) influences neurite outgrowth

and orientation (Jaffe & Poo, 1979; Patel & Poo, 1984). Neurite outgrowth is facilitated in the direction of the cathode (Jaffe & Poo, 1979; Patel & Poo, 1984) and inhibited from the direction of the anode when weak direct current electrical fields are applied across medulla fragments of chick embryos (Marsh & Beams, 1946), DRG of chick embryos (Jaffe & Poo, 1979) and embryonic *Xenopus* neurons (Patel & Poo, 1982). Electric fields appear to increase both the number of neurites per neuron in cell cultures and the average length of the neurites per neuron (Pomeranz *et al.*, 1984; Sisken & Smith, 1975).

The promising effects of electrical fields on neurite outgrowth *in vitro* studies further supports the idea that electrical fields might enhance axonal regeneration of peripheral nerves and functional recovery *in vivo* studies. Pomeranz *et al.* (1984) demonstrated that ES facilitates axonal sprouting of the intact saphenous nerve in adult rats with transected sciatic nerves. Weak direct current (DC) electrical fields were applied for 21 days (6 days on and 1 day off), 30 mins per day, to the intact saphenous nerve. Based on histological data, which correlated with hind limb reflexes, both the application of weak DC (1 μA) fields (with cathode located distal to the lesion) and the application of stronger AC electrical fields (1000 μA per pulse) significantly enhanced the sprouting of intact saphenous nerves compared to those within the controls. This is in agreement with a histological study, which concluded that the application of DC electrical fields accelerated the rate of axonal regeneration (Kerns *et al.*, 1986). Although the aforementioned studies suggests that the application of electric fields increases the number of regenerating axons in the distal nerve stump (Pomeranz *et al.*, 1984) and accelerates the rate of recovery in comparison to controls (Kerns *et al.*, 1986), these studies did not examine the relative positions of the electrodes (i.e., cathode and anode) across the nerve (Politis *et al.*, 1988). This was investigated in a study conducted by Politis *et al.* (1988). The study evaluated the effects of ES on the early rate of axonal regeneration. Direct current ES (1.5 μA) was applied continuously, via a nerve cuff, to a transected and re-sutured frozen rat sciatic nerve for

6, 12, and 18 days. Axonal regeneration was quantified by counting the number of antibodies of neurofilament protein in the distal nerve stump. The rate of early axonal regeneration was found to accelerate only when the cathode was placed distally to the injury site. Conversely, when the anode was placed distally to the injury site fewer axons were found in the distal stump compared to the control group. These results suggest that weak DC electric fields enhance axonal regeneration by orienting the axons and promoting axonal regeneration towards the target when the cathode is in that direction. Regenerating axons favorably grow towards the cathode. Therefore, the cathode should be placed distal to the injury site in studies examining the effects of ES on axonal regeneration and functional recovery. Placing the cathode distal has become standard protocol and it was used in our study.

With the cathode placed distal, constant low-intensity ES (10 μA) was applied to transected adult rat sciatic nerves for 3 weeks to evaluate its effect on axonal regeneration (Roman *et al.*, 1987). Histological and electron microscopy data indicated an increase in both the number of axons and blood vessels in the cross-section of stimulated nerves compared to controlled nerves. In a similar study, crushed sciatic nerves of rats were continuously electrically stimulated (1 μA) for 3 weeks (Mendonca *et al.*, 2003). Based upon histologic and morphometric data, ES increased the blood supply by augmenting the number and diameter of the blood vessels supplying the nerves. ES also appeared to enhance axonal regeneration and functional recovery, indicated by a significant improvement in the sciatic functional index (SFI), which is an indirect measurement of the motor function of the hind paws of rats. The positive effects of ES on axonal regeneration and functional recovery have also be shown in studies on crushed/compressed peripheral nerves of rats (Pockett & Gavin, 1985; Roman *et al.*, 1987; Al Majed *et al.*, 2000), mice (Ahlborn *et al.*, 2007), rabbits (Nix & Hopf, 1983), canines (Rozman *et al.*, 2000) and humans (Amirjani, 2005). Evidence of ES accelerating recovery in crushed/compressed nerves includes the following: the toe spread reflex (splaying of the toes

when a rat is picked up by the tail) recovered faster in rats with ES than controls (Pockett & Gavin, 1985), tetanic tension, twitch forces, and muscle action potential returned to pre-injury values in rabbits faster than those of the controls (Nix & Hopf, 1983), and sensory NC values and terminal motor latency improved faster compared to controls in humans with CTS (Amirjani, 2005).

In a study conducted by Nix and Hopf (1983), the motor nerve innervating the soleus muscle was crushed in rabbits. A day later ES was applied for 4 weeks continuously, at a low frequency (4 Hz) and a low intensity (1.5 times motor threshold before injury), to the motor nerve proximal to the injury site. Twitch-force, tetanic-tension, and muscle action potential amplitudes were assessed every week with supramaximal ES. Consequently, the treatment group (i.e., receiving ES) showed a significantly faster recovery of motor function, attaining pre-operative values 1 week earlier, than the control group. Therefore, ES appeared to enhance the rate of axonal regeneration and motor recovery as evaluated by the significantly faster improvement in motor function recovery in the treatment group.

ES also accelerated axonal regeneration in crushed rat sciatic nerves (Pockett & Gavin, 1985). ES was applied to the nerve proximal to the injury site, after different time delays (25 min – 1 h) and at different durations (15 min – 1 h). The ES was applied at 1 Hz, at intensity just above motor threshold. The toe spray reflex recovered faster in the treatment group (at ~ 4 days) than in the control group (at ~10 days). ES was found to be the most effective at accelerating axonal regeneration when applied immediately following nerve injury, but still produced positive effects when application was delayed up to 1 h. The most effective duration of ES application for the recovery of the toe-spray reflex was determined to be 1 h.

Accordingly, Al-Majed *et al.* (2000) determined that 1 h of ES was just as effective at producing a significant positive impact on axonal regeneration as applying ES for a longer period of time. ES was applied immediately to the proximal nerve stump of transected and re-

sutured rat femoral nerves (at 20 Hz and supramaximally) at different durations of 1 h - 2 weeks. The motor neurons that regenerated axons into the cutaneous and the motor branches were backlabelled with retrograde neurotracers. Axonal regeneration was evaluated at 2, 3, 4, 6, 8, and 10 weeks post-operatively. ES was concluded to accelerate axonal regeneration by compressing staggered regeneration, for it took less time for all the motor axons to cross the injury site (3 weeks vs 8 - 10 weeks). ES also promoted preferential growth of regenerating motor axons into appropriate muscle pathways (i.e., PMR). Only 1 h of ES was shown to produce these same results.

Due to the efficacy of ES in rat (Mendonca *et al.*, 2003; Roman *et al.*, 1987; Al Majed *et al.*, 2000; Politis *et al.*, 1988; Pomeranz *et al.*, 1984; Pockett & Gavin, 1985), mice (Ahlborn *et al.*, 2007; Geremia *et al.*, 2007), canine (Rozman *et al.*, 2000), and rabbit studies (Nix & Hopf, 1983), the possibility of using ES as a clinical treatment in humans with chronic nerve compression was explored. In Amirjani (2005), constant low-frequency (20 Hz) ES was applied at a maximum tolerance to the median nerve at the site of injury for 1 h immediately following CTS decompression surgery in individuals with moderate or severe CTS that had substantial axonal loss and no conduction block. The study demonstrated that even a brief amount of ES enhances axonal regeneration, as indicated by a significant difference in the estimated number of motor units in the median-innervated thenar muscles compared to baseline at 6 – 8 months post-operatively (Amirjani, 2005). In addition, ES appeared to significantly enhance the recovery time of the sensory CV, SNAP amplitude, and terminal motor latency, as indicated by a faster return to pre-operative values compared to the control group. However, ES was not enough to significantly improve hand function recovery or outcome. ES was applied at the site of injury, where local anesthesia from surgery may have blocked some of the median nerve fibers. This may have prevented the entire nerve from being depolarized and resulted in the suboptimal results.

I – VII THESIS:

1. Objectives:

The purpose of this thesis is of 3 fold. First, given the importance of having a reliable means to estimate the severity of motor axonal loss in the investigation of peripheral nerve trauma in human subjects, we start by investigating the feasibility of increasing the sample size of single motor unit action potentials using surface electrical stimulation on the median nerve. The results are reported in chapter 2. Second, we wish to further test whether having a larger sample of single motor unit action potentials using this modified technique would improve the reliability of the MPS method. Results on that study are reported in chapter 3. Lastly, using the MPS method, we ask the question of whether 1 h of post-surgical, low-frequency (20 Hz) ES, delivered immediately at a site proximal to the nerve injury (median nerve at the antecubital fossa) following CTS decompression surgery, would enhance axonal regeneration and improve functional recovery and outcome (i.e., manual dexterity).

2. Hypotheses to be Tested:

In the first two studies, we hypothesize that a larger single motor unit action potential sample can be obtained by the additional use of a long stimulus pulse width. Furthermore, the increase in sample size would enhance the test-retest reliability of the MPS methods. In the last study, we hypothesize that ES will significantly enhance axonal regeneration, as indicated by a significant increase in MUNE in the treatment group compared to the control group. The NC parameters and scores on the Levine self-assessment questionnaire will show significant improvement faster in the treatment group than the control group, suggesting ES significantly improves recovery. Performance on the hand function tests (the Purdue pegboard subtest and the Moberg pick-up test) are hypothesized to significantly improve faster and to a greater extent

in the treatment group versus the control group, indicating ES significantly improves functional outcome.

3. Rationale:

In moderate and severe CTS there can be substantial axonal loss; it is a good model of axonal degeneration. CTS was chosen for our study for a number of reasons. First, the condition is extremely common (10% lifetime risk; Stevens *et al.*, 1988). Therefore, it was easy to recruit subjects. Second, the distance that the nerve had to regenerate was relatively short compared to more proximal nerve injuries (e.g ulnar nerve compression at Guyon's canal at the antecubital fossa). Third, the rate of axonal regeneration is faster and results in better functional outcome in compressed injuries, such as CTS, than transected ones like brachial plexus injuries. Consequently, the results of our study could be assessed within months instead of years. Finally, established non-invasive methods for estimating the amount of axonal regeneration for the median-innervated thenar muscles exist, allowing an easier and more reliable evaluation of the treatment technique proposed in our study.

Amirjani (2005) also used CTS as a model of axonal degeneration and investigated the effects of post-surgical ES on the rate of axonal regeneration and improvement in hand functionality. ES was delivered (100 μs duration, continuous 20 Hz train, at maximum tolerance limit) immediately after CTS decompression surgery at the site of incision in 24 symptomatic hands. There was an enhancement in motor axonal regeneration, as indicated by a significant increase in the estimated number of motor units compared to baseline ($p < 0.05$) 6 – 8 months post-operatively in the treatment group. However, with the exception of sensory nerve CVs, SNAP amplitudes and terminal motor latencies, which significantly improved earlier in the treatment group (ES received after CTS decompression surgery) in comparison to the control group (only underwent surgery), there were no significant differences on outcome measures

between the control and treatment groups. The suboptimal results may be due to the application of a local anesthetic (i.e., lidocaine) at the site of incision during surgery, where the ES was subsequently delivered. Lidocaine is a potent sodium channel antagonist (Fukuda *et al.*, 2005). Therefore, some of the sodium ion channels were blocked, preventing the ES from depolarizing all of the nerve fibers. Application of ES more proximal to the site (i.e., antecubital fossa) is anticipated to depolarize the nerve fibers more effectively and produce significant results. The antecubital fossa is an ideal location to deliver ES, for it is far enough away from the sodium channel blockade that the nerve fibers would not be affected. Also the median nerve is fairly superficial at this location and there are no major nerves nearby, facilitating its localized activation without co-activating other nerves.

In our study, 1 h of continuous, low-frequency (20 Hz) post-surgical ES was delivered to the injured nerve. This duration and intensity were based on past studies that showed an improvement in axonal regeneration in rats, (Al Majed *et al.*, 2000) and humans (Amirjani, 2005), with only 1 h of low-intensity (20 Hz) ES. Based on Amirjani (2005), the maximum tolerable stimulus intensity level for each subject was used (10 - 20 mA). This is in contrast to animal studies that typically use supra-maximal stimulation.

In our study, the sample size (15 subjects, 20 symptomatic hands) was a large enough sample size to detect significant results between the groups. In a similar study (Amirjani, 2005) a sample size of 20 subjects (24 symptomatic hands) was used. Possible confounding factors are age, type of nerve trauma, activities ensuing surgery, and elapsed time between injury and surgery. To control for possible age and nerve trauma biases the mean, ages of the subjects were kept relatively similar in each group, and all the subjects had moderate or severe CTS with substantial axonal loss and an absence of conduction block.

Our study was the first to investigate the effect of ES on axonal regeneration and functional outcome when ES was applied to the median nerve proximal to the injury site following CTS decompression surgery. If brief ES does improve axonal regeneration and

functional outcome, this technique could be routinely applied in the clinical setting because it has been previously found to be relatively painless, fast, safe, and easy (Amirjani, 2005). This procedure could be a useful model for examining the application of post-surgical ES in other peripheral nerve injuries.

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Seddon	Sunderland	Function	Pathological Basis
Neurapraxia	**Type 1**	Focal conduction block. Mainly motor function and proprioception affected.	Local myelin injury, primarily large fibers. Axonal continuity.
Axonotmesis	**Type 2**	Loss of nerve conduction at injury site and distally.	Disruption of axonal continuity with Wallerian degeneration. Intact endoneurial tubes, perineurium, and epineurium
Neurotmesis	**Type 3**	Loss of nerve conduction at injury site and distally.	Loss of axonal continuity and endoneurial tubes. Intact perineurium and epineurium.
	Type 4	Loss of nerve conduction at injury site and distally.	Loss of axonal continuity, endoneurial tubes and perineurium. Intact epineurium.
	Type 5	Loss of nerve conduction at injury site and distally.	Transection of the entire nerve.

Table 1-1. Comparison of the Seddon and Sunderland classification system for nerve injuries. Modified from Lundburg G: Nerve Injury and Repair. Edinburgh, Churchill Livingstone, 1988.

CTS Severity	NC Study Values
Mild	Transcarpal sensory CV < 50 m/s & TML ≤ 4.2 ms
Moderate	Transcarpal sensory CV < 50 m/s & TML > 4.2 ms
Severe	Absence of SNAP & TML > 4.2 ms

Table 1-2. Carpal tunnel syndrome (CTS) severity based upon standard nerve conduction (NC) study criteria. CV stands for conduction velocity, TML stands for terminal motor latency, and SNAP stands for sensory nerve action potential.

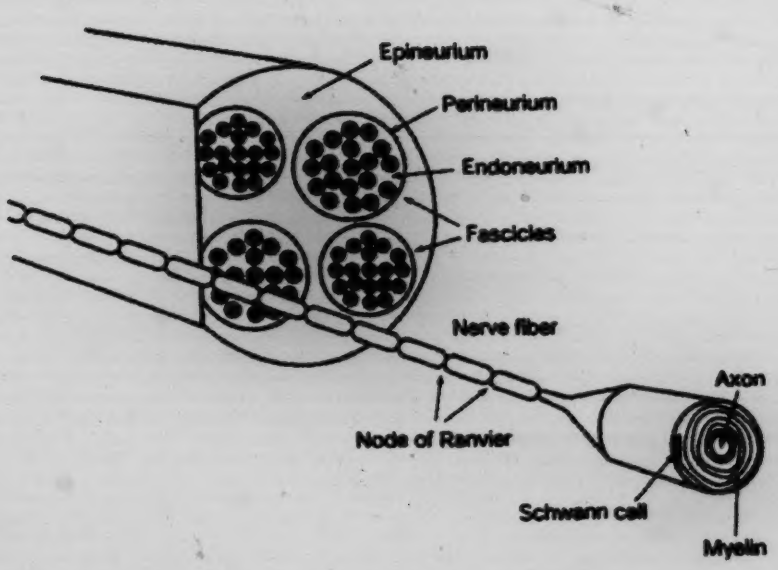

Figure 1-1. Normal anatomy of nerves. The connective tissue and neural fiber components of nerves. (Adapted from: Moller AR. Neural Plasticity and Disorders of the Nervous System. Cambridge: Cambridge University Press; 2005).

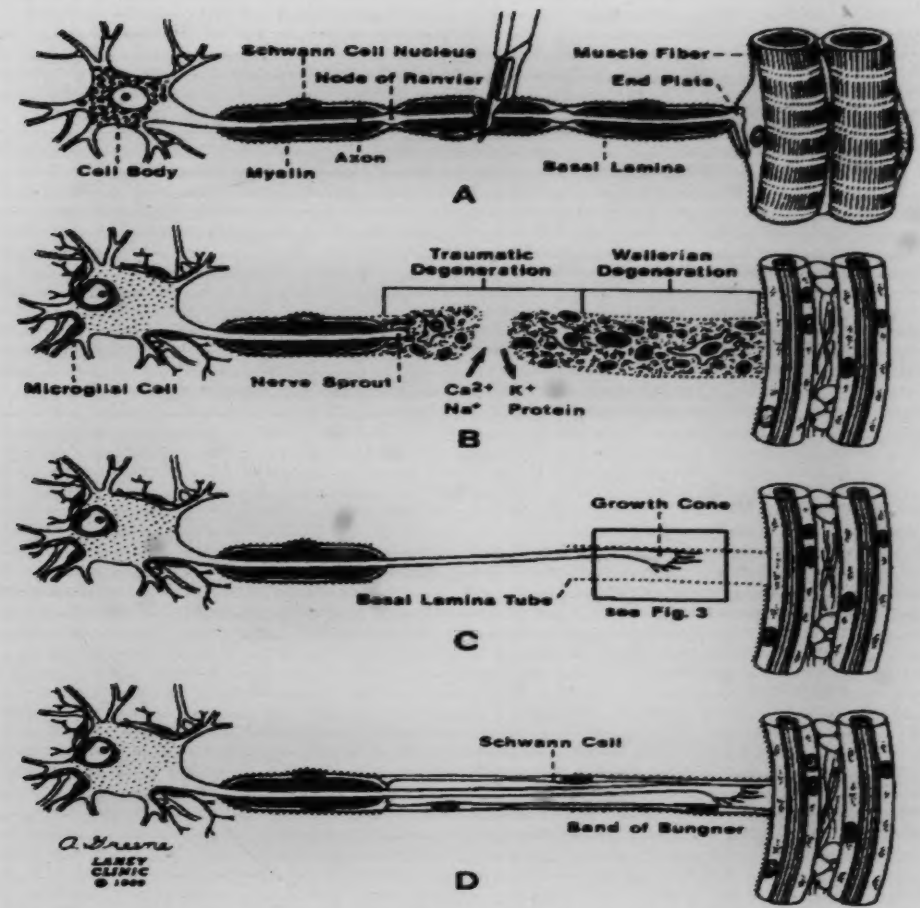

Figure 1-2. Peripheral axonal regeneration. (A) Diagrammatic representation of a normal soma and processes. A typical peripheral nerve will contain thousands of such axons. (B) Traumatic and Wallerian degeneration. (C) Regeneration. Note axonal growth cone growing into empty basal lamina. (D). Ensheathment. Note SC alignment around axon to form bands of Bungner. Degree of myelination will be determined by the type of axon (Modified from Lahey Clinic).

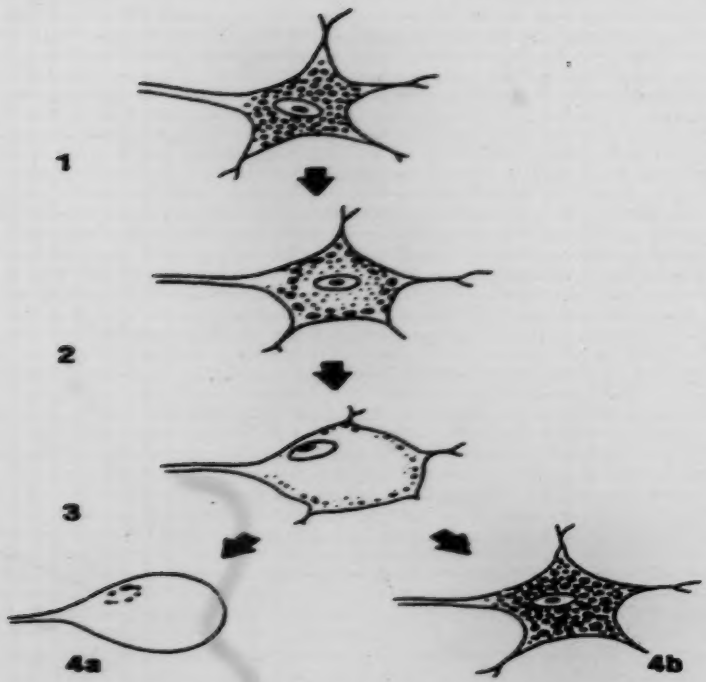

Figure 1-3. Chromatolysis: Reaction of soma to injury of its axonal extension.
1. Normal nerve cell prior to trauma with even distribution of Nissl substance. 2. By 48 hours following nerve injury, the Nissl substance is disappearing and changing into "dust particles." 3. At 2 or 3 weeks, the cell is swollen with an eccentrically located nucleus and only a marginal appearance of the Nissl substance. The nucleolus is also eccentrically placed in the nucleus. Once the cell has reached this stage, it can proceed in one of two directions. 4a. The cell can die and at first appear as a ghost cell and then completely disappear. 4b. It is also possible for the cell to recover and again form discrete Nissl bodies. Also, the cell as a whole is no longer swollen and the nucleus is again centrally located. (Modified from Bots G Th: Pathology of nerves. In: Vinken PJ, Bruyn GW: Handbook of Clinical Neurology, Vol 7, Diseases of Nerves (Part I). Amsterdam, North-Holland, 1970, pp. 197-243.

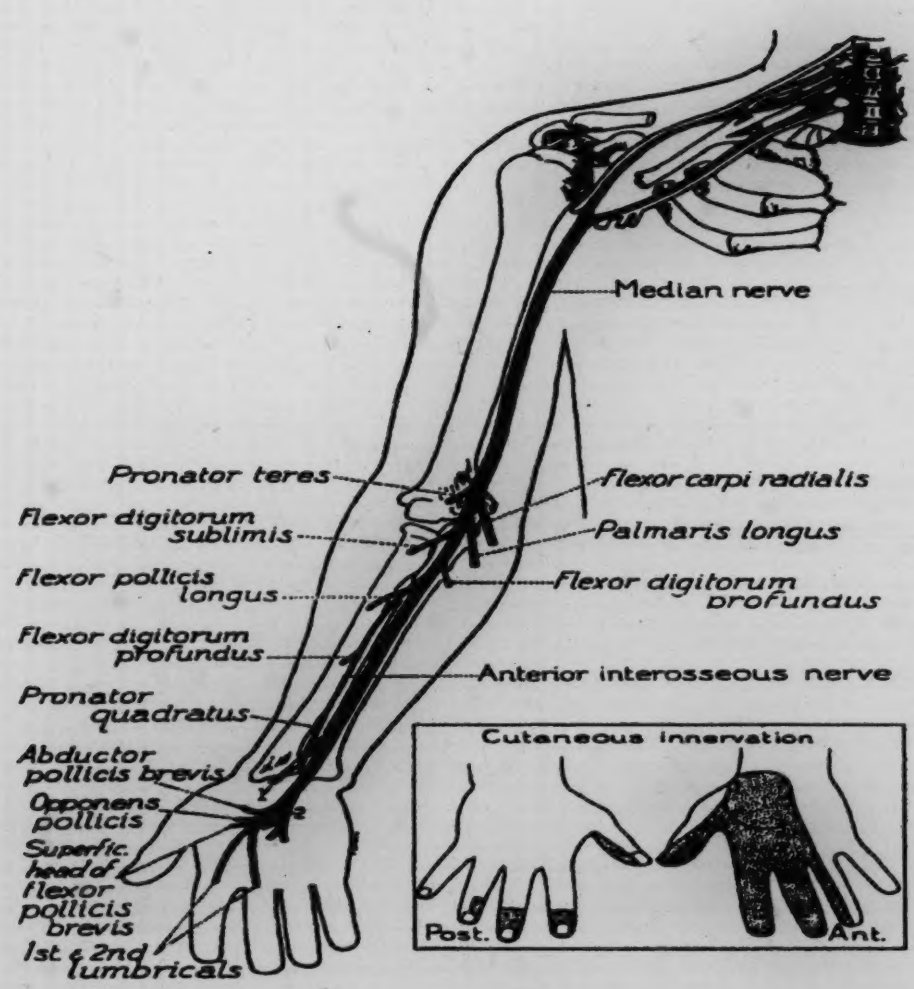

Figure 1-4. Median nerve course and neural branching in the extremity. Note the cutaneous innervation of the anterior and posterior portions of the hand (inset). (From Haymaker W, Woodhall B: Peripheral Nerve Injuries. Philadelphia, W.B. Saunders, 1953).

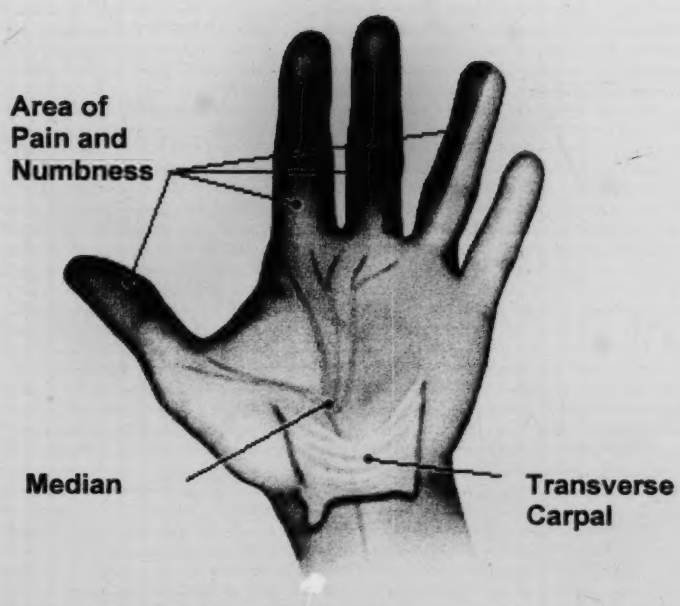

Figure 1-5 Median Nerve Distribution.
The areas of the hand the median nerve typically innervates. Taken from Medicine Net, Inc.
(http://images.medicinenet.com/images/illustrations/carpal_tunnel.jpg)

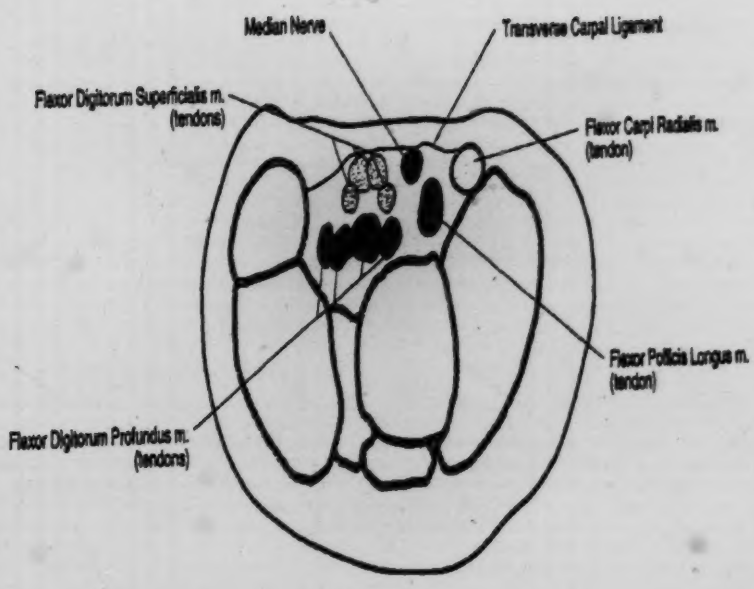
Median Nerve
Transverse Carpal Ligament
Flexor Digitorum Superficialis m. (tendons)
Flexor Carpi Radialis m. (tendon)
Flexor Pollicis Longus m. (tendon)
Flexor Digitorum Profundus m. (tendons)

Figure 1-6. Cross-sectional view of the carpal tunnel and its contents at the level of the first row of carpal bones. (From Hennessey WJ & Kuhlman KA (1997). The anatomy, symptoms, and signs of carpal tunnel syndrome. *Physical Medicine and Rehabilitation Clinics of North America* **8**, 439-457.

Chapter 2

Improving the Accuracy of the Multiple Point Stimulation Technique

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2 – 1. Introduction:

Motor unit number estimation (MUNE) is a useful technique for diagnosing and monitoring the progression of diseases in which motor neuron or axonal degeneration is a major feature. Multiple point stimulation (MPS) is one of the most widely used MUNE techniques. Like other MUNE methods, the estimate is derived by comparing the size of the average surface motor unit action potential (SMUP) to the size of the maximum compound muscle action potential (CMAP; Doherty *et al.*, 1995). Although obtaining the maximal CMAP is relatively simple, deriving an average SMUP that is truly representative of the constituent motor units in the muscle is more difficult. An adequate sample size and unbiased activation of motor units of different sizes are important factors that influence the accuracy and test-retest reliability of the MUNE results (Blok *et al.*, 2005). A minimum of 12-15 SMUPs is required (Bromberg, 2002). However, in practice this is sometimes difficult to attain, especially in obese patients, or nerves that cannot be readily accessed percutaneously. Therefore, means that can help to increase the yield of SMUPs should improve the accuracy and reliability of the results.

One possible method to increase the SMUP sample size, as suggested by a number of theoretical studies, is by varying the stimulus pulse width (Szlavik & de Bruin, 1999). The objective of this study was to determine whether using stimulus pulses of different widths at each location along the nerve would substantially increase the sample size of SMUPs.

2 – II. Methods:

1. Subjects:

The experiment was conducted on 10 healthy volunteers of equal gender distribution, age 25±8 years old [mean±SD] with the approval from the Human Ethics Board at the University of Alberta.

2. Motor Unit Number Estimation (MUNE):

The MPS MUNE technique was performed on the median-innervated thenar muscles, using an Advantage Electromyography (EMG) machine (Neuroscan Inc., VA). The signals were band pass filtered between 5 Hz and 2 kHz and sampled at 5 kHz. The skin was gently abraded and cleansed with rubbing alcohol. Disposable surface (Ag/AgCl) EMG strip electrodes (1 x 2.5 cm) were used. The active electrode was placed over the motor point of the median nerve innervated thenar muscles, whereas the reference electrode was positioned distally at the metacarpal-phalangeal joint of the thumb. A ground electrode was attached to the dorsum of the hand. Hand temperature was maintained between 32 and 36 °C. Using a hand-held bipolar constant current stimulator, course of the median nerve was mapped out between the wrist and distal forearm, and from the antecubital fossa to the axilla. When localizing the median nerve in the upper arm, co-activation of the ulnar nerve was avoided by asking the patients to indicate if any sensation was felt in their 5^{th} digit. The maximal CMAP was obtained by stimulating the median nerve just proximal to the distal wrist crease. SMUPs were collected at multiple points along the superficial course of the median nerve by finely graded threshold stimulation. Inclusion criteria for SMUPs were reproducible all-or-none response, and no alternation. SMUPs were acquired with pulse widths of 0.05 ms and 1 ms. Following SMUP collection, the custom software program averaged the SMUPs in a "data point by data point" manner. MUNE was calculated by dividing the peak-to-peak amplitude of the maximal CMAP by that of the average SMUP.

The data was analyzed using SPSS 12 (SPSS, Chicago, Illinois) and descriptive statistics are reported as mean±SD.

2 – III. Results:

MPS is typically performed using short pulse duration of 0.05 ms. In this study the additional use of a long pulse width of 1 ms substantially increased the yield of SMUPs. All subjects had normal MUNE results (357 motor units ±134). Figure 2-1 illustrates data from a representative subject.

We found that the 1 ms stimuli activated different motor axons than those found at short pulse width (0.05 ms). Table 2-1 summarizes the number of SMUPs collected from each subject at the two pulse widths. The percentage increase indicates the number of SMUPs collected when the wider pulse width was used in combination with the narrower pulse width. The mean percentage increase was 35±23 %.

Although it can be clearly seen that this method substantially increased the yield of SMUPs, a crucial question is whether there was any bias in the size of the SMUPs collected using long pulse width. This was evaluated by plotting the size distribution of the SMUPs obtained in the same representative subject (Fig. 2-2).

The skewness and range of distribution in figures 2-2A and 2-2B are very similar. The same trends also applied to the other 9 subjects. Figure 2-3 shows the cumulative group results. An interesting observation was the activation of H-reflexes with long width pulses (represented by the asterisks in Fig. 2-3B). No H-reflexes were found at the narrower pulse duration.

The SMUPs found at the wrist were compared with those collected in the upper arm. The percentage increase in the number of SMUPs found with 1 ms stimuli ranged from a 0 to 88% increase at the wrist (35±21%), and from an 8 to 38% (mean of 23±8.5%) increase in the upper arm. Although the increase of SMUP yield was slightly larger in the wrist than at the upper arm, the difference was not statistically significant.

2 – IV. Discussion:

In this study, we demonstrated that when a stimulus of long pulse width (1 ms) was used in combination with a short pulse width (0.05 ms) during MPS, it resulted in a significant increase in the number of SMUPs obtained.

Although the effects of pulse width on motor axonal activation have been studied, the results may not be applicable to the experimental paradigm of MPS. For example, by modeling the cable properties of motor axon, Grill & Mortimer (1996) suggested that different motor units could be selectively recruited by altering the stimulus pulse width because myelinated nerve fibers of different sizes have different thresholds. However, in that model, it was assumed that the axon was situated in a homogenous medium and the stimulus point source was located immediately adjacent to the nerve. These assumptions are likely not applicable to surface stimulation where there are interposed skin, subcutaneous tissues, tendons and blood vessels between the stimulator and the nerve. Indeed, Szlavik & de Bruin (1999) investigated the effect of pulse widths on the nerve fibers recruited using the incremental stimulation technique. In that study, the median nerve was stimulated at a single location proximal to the elbow. Although at 0.05 ms, the shorter pulse width used, a slightly greater number of large diameter motor axons were activated, the magnitude of that was much smaller than what was predicted by their computational model. This apparent discrepancy is understandable considering that the stimuli used in the model were located within several mm of the nerve without any interposed tissue in between. That assumption is likely not applicable to surface stimulation used in the incremental stimulation technique.

Taking the above considerations into account, the lack of selective bias found in this study is perhaps not unexpected. A likely reason is that with the MPS technique, instead of stimulating the nerve at a single location, the ensemble of SMUPs are collected by stimulating the median nerve at a large number of accessible sites

throughout the wrist and the upper arm. In that scenario, the cable properties of the motor axons are not the only factor at play. Rather, differences in the amount and density of interposed tissues and relative depth of the motor axons in the fascicle at each location likely have a bigger role in determining which axon can be most easily activated. In fact, using a simulation model that took these assumptions into account, (Major & Jones, 2005) showed that preferential activation of large diameter motor axon is unlikely to occur in MPS.

Although combining the 1 ms pulse width helped to increase the SMUP sample size, a caveat is that at the longer pulse width, more alternation of SMUPs was observed, excitability thresholds of neighboring axons became compressed and occasionally even reversed. These observations are in agreement with Gorman & Mortimer's study (1983) in which they found threshold separation between adjacent nerve fibres was greater at shorter pulse widths. To circumvent this difficulty when using long width pulses, more stimuli need to be delivered at each location to avoid erroneous inclusion of compound SMUPs.

Some SMUPs collected with the wider pulse width were H-reflexes, whereas no H-reflexes were found with the narrow pulse width. Preferential activation of sensory fibres that occurs with longer pulse width is well described (Panizza *et al.*, 1998). A possible explanation for this is the recent finding of greater percentage of persistent sodium channels in sensory nerve fibres (Bostock & Rothwell, 1997). The difference in persistent sodium channels affects the strength-duration time constant (Bostock & Rothwell, 1997) and rheobase (Mogyoros *et al.*, 1996) of nerve fibres—both important determinants of nerve fibre excitability.

Although we demonstrated a substantial increase in the sample size of SMUPs by also using a long pulse width, it will be important to test whether this new test

procedure will indeed result in greater test-retest reliability of the motor unit number estimates.

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Mogyoros, I., Kiernan, M. C., & Burke, D. (1996). Strength-duration properties of human peripheral nerve. *Brain* **119**, 439-447.

Panizza, M., Nilsson, J., Roth, B. J., Grill, S. E., Demirci, M., & Hallett, M. (1998). Differences between the time constant of sensory and motor peripheral nerve fibers: Further studies and considerations. *Muscle & Nerve* **21**, 48-54.

Szlavik, R. B. & de Bruin, H. (1999). The effect of stimulus current pulse width on nerve fiber size recruitment patterns. *Medical Engineering & Physics* **21**, 507-515.

Subject	0.05 ms	1 ms	Total # of SMUPs	% Increase
1	13	12	25	92
2	22	14	36	64
3	22	6	28	27
4	21	8	29	38
5	18	4	22	22
6	18	4	22	22
7	18	4	22	22
8	20	7	27	35
9	20	4	24	20
10	21	4	25	19

Table 2 -1. Number of SMUPs collected at 0.05ms, 1ms, and in total for each subject. The percentage increase indicates the percentage of additional SMUPs collected in each subject by also using the 1 ms pulse.

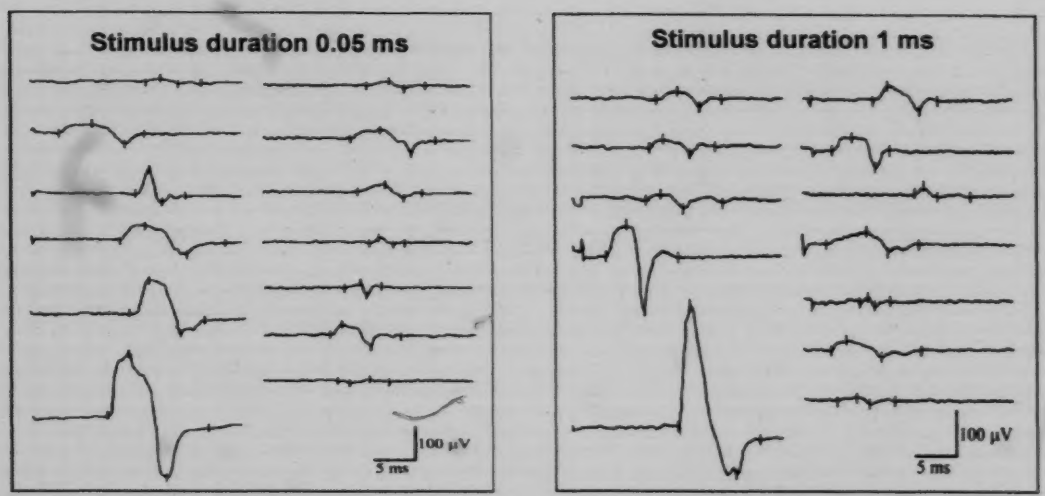
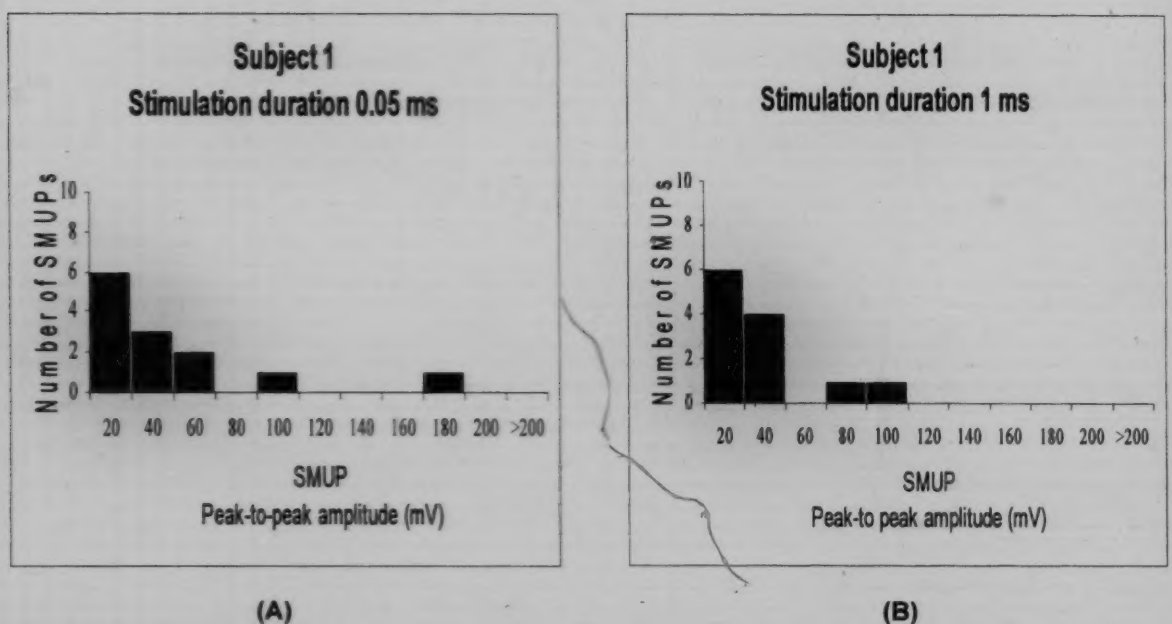
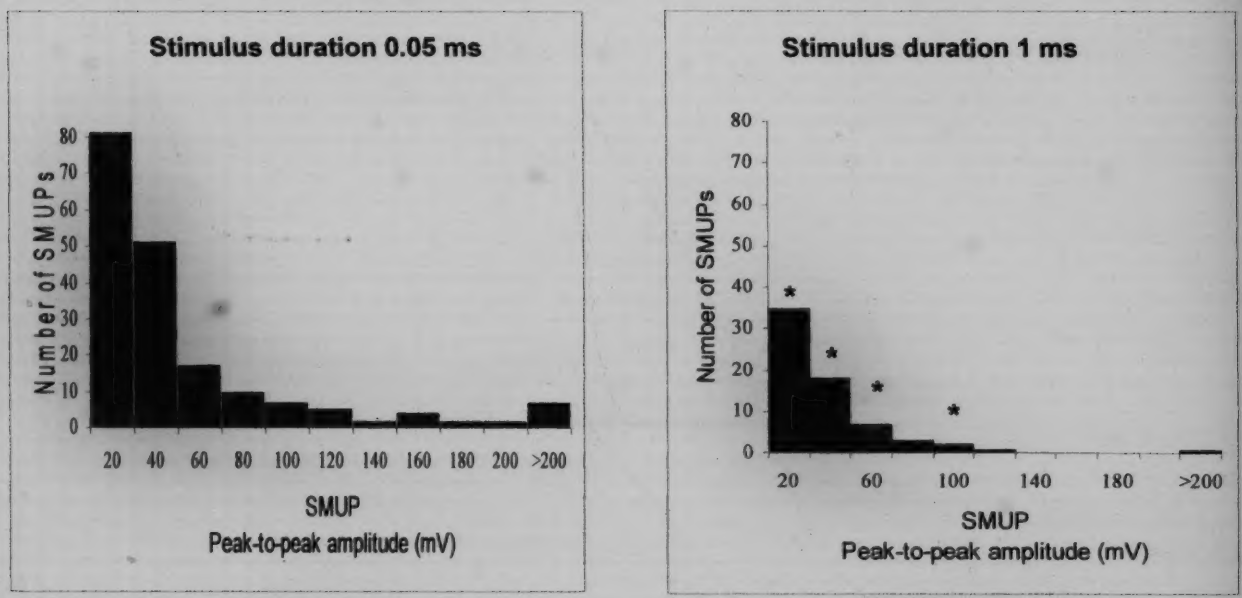


Figure 2-1. Original surface motor unit action potentials (SMUPs) obtained in a representative subject with stimulus pulse width durations of 0.05 ms (A) and 1 ms (B). There was no substantial difference in the size of the SMUPs obtained through different pulse widths.



(A) (B)

Figure 2-2. Typical distribution of the sizes of the surface motor unit action potentials collected at the narrow pulse width (0.05 ms) (A) and wide pulse width (1 ms) (B) in a representative subject.



(A) (B)

Figure 2-3. The distribution of surface motor unit action potential (SMUPs) collected with stimulation durations of 0.05ms (A) and 1ms (B) from all 10 subjects. Each asterisk in figure 2-3B denotes a motor unit action potential activated through H-reflex.

Chapter 3

Test-Retest Reliability of a Modified Multiple Point Stimulation Technique for Motor Unit Number Estimation

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3 – I. Introduction:

Motor unit number estimation (MUNE) is a neurophysiological method that can help to define the severity, follow the progression and detect the efficacy of treatment of neuromuscular diseases in which motor neuronal or axonal loss is a major feature (Doherty & Brown, 1993; Simmons *et al.*, 2001). There are a variety of MUNE techniques, most of which derive the estimate of motor units from the ratio of the maximum compound muscle action potential (CMAP) to the average surface motor unit action potential (SMUP). To be clinically useful, the method must have sufficiently high test-retest reliability with no sampling bias. Major contributors to high MUNE reproducibility are the sample size of SMUPs collected and the number of motor axons within the nerve (McComas, 1991). One assumption of MUNE is that the sample of SMUPs is representative of the whole population. SMUP sizes range considerably within a muscle. Therefore, it is important to collect a large sample size of SMUPs (McComas, 1991). All MUNE techniques share the same challenge of acquiring an adequate sample of SMUPs that accurately represents all the motor units within a muscle (Blok *et al.*, 2005; Bromberg, 2007).

Multiple point stimulation (MPS) is one of the most commonly used MUNE techniques (Lomen-Hoerth & Olney, 2000; Doherty *et al.*, 1995). In standard MPS, SMUPs are collected along the superficial portion of a nerve using only short pulse width. Doherty and Brown (1993) showed that the mean percentage error of the MUNE could be reduced if more than 10 SMUPs were obtained. In a recent study, we demonstrated that a greater increase in the sample size of SMUPs could be achieved by additionally using a long stimulus pulse width (1 ms; Porter *et al.,in press*). However, whether that would result in higher test-retest reliability has not been investigated. The

objective of this study was to test the hypothesis that this modification would substantially increase the within subject test-retest reliability of MPS.

3 – II. Methods:

1. Subjects:

The experiment was conducted on 11 young, healthy volunteers [6 males; 27±3 years old (mean±SD)] and 5 elderly, healthy volunteers (2 males; 71±11) with approval from the Human Ethics Board at the University of Alberta.

2. Motor Unit Number Estimation (MUNE):

Modified MPS was performed on the median nerve innervated thenar muscles by the same experimenter on separate days. The amplified EMG signal was digitalized using an A-D converter card (National Instruments, San Antonio, Texas) in a PC computer running LabVIEW 5.0 custom written program (National Instruments). The signals were band pass filtered between 5 Hz and 2 kHz and sampled at 5 kHz. The skin was gently abraded and cleansed with rubbing alcohol. Disposable surface (Ag/AgCl) EMG strip electrodes (1 x 2.5 cm) were used. The active electrode was positioned over the motor point of the median nerve innervated thenar muscles and the reference electrode over the metacarpophalangeal joint of the thumb with the ground electrode attached to the dorsum of the hand. The thumb was immobilized by tape in an adducted position. Hand temperature was maintained between 32 and 36°C by using a heat pack. The course of the median nerve was mapped between the wrist and distal forearm, and from the antecubital fossa to the axilla, using a 2 cm high, flat low profile hand-held bipolar stimulator with saline soaked felt tip electrodes. Co-activation of the ulnar nerve was avoided by having patients indicate if any sensation was felt in their 5th digit. The maximal CMAP was acquired by supramaximally stimulating the median nerve just

proximal to the distal wrist crease. The superficial course of the median nerve was stimulated at consecutive sites from the distal wrist crease to the distal forearm and then from the antecubital fossa to the axilla, by using finely graded threshold stimulation to activate single motor axons. SMUPs, defined as all-or-none responses that occurred in a reproducible and orderly fashion, evoked with distinct thresholds, were collected at each site. Features in the program that allowed the lowest threshold SMUPs to be reliably identified included: superimposition and editing capability of all stimulated responses and template subtraction of successively higher threshold SMUPs. In most instances, only the first 1 or 2 lowest threshold SMUPs were collected, thereby circumventing the difficulties associated with alternation.

In the first 10 subjects, the lowest threshold SMUPs were first evoked with the conventional pulse width of 0.05 ms, followed by 1 ms. To ascertain whether the order of administration had an impact on the MUNE results, in the remaining 6 subjects the stimulus pulse widths were randomized. This was accomplished by using a random number generator (SPSS 12, Chicago, Illinois). To establish the practicality of applying this modified technique in the clinical setting, the extra time required to capture additional SMUPs with the 1 ms stimulus pulse width was measured with a stop watch.

To avoid duplication, if the same SMUP was activated with both pulse widths, only the one activated with the conventional 0.05 ms pulse width was kept. Position of the stimulator was carefully adjusted to find the optimum orientation to discretely activate SMUPs with distinct stimulus thresholds. To ensure maximal stability of the stimulator over the nerve, it was securely tied down with a Velcro strap looped around the subject's arm. The Velcro strap was so adjusted that it prevented inadvertent displacement of the stimulator, but yet not so tight that it indented the skin or induced ischemia and discomfort. Once the lowest threshold SMUPs had been collected, the stimulator was moved approximately 15 mm along the nerve trunk. This distance was so chosen

because, beyond that, the risk of inadvertently activating the same SMUPs is negligible (Doherty & Brown, 1993). After all SMUPs were collected (i.e., no additional SMUPs were observed at either stimulus pulse width), the program averaged the SMUPs in a "data point by data point" manner. The MUNE was calculated by dividing the negative peak amplitude of the maximal CMAP by that of the average SMUP. MUNE based on the standard MPS method only included SMUPs captured using the conventional 0.05 ms pulse width, while results obtained by the modified technique included all SMUPs captured by both pulse widths. Test-retest reliability of these two methods was computed for the young subjects and the elderly subjects separately using the intraclass correlation (ICC) method. In comparison with Pearson's correlation analysis, ICC is more vigorous because it takes both random and systemic errors into account. A Cronbach's α value of 1 represents perfect correlation. The data was analyzed using SPSS 15 (SPSS, Chicago, Illinois). Descriptive statistics are reported as mean±SD. *A priori* the statistical significance was set at $p<0.05$. To detect whether there was any sampling bias with each of the stimulus pulse width, sizes of the SMUPs obtained using the standard and modified MPS method were compared using paired sample student's t test.

3 – III. Results:

1. Sample Size and Potential Sampling Bias:

Compared with the standard MPS method, there was a substantial increase in the number of SMUPs collected, from 18±3 to 23±4, in the young subjects using the modified MPS technique (Table 3-1). In the elderly subjects there was an increase from 16±3 to 20±3 motor units using the modified MPS technique.

To address the question of whether there was any bias in the sizes of the SMUPs obtained using the two different pulse widths, we tabulated the SMUP negative-peak amplitudes for all subjects using the modified and standard MPS methods (Table

3-2). No significant difference between SMUPs activated by using the standard or modified method was found. At test 1 for the young subjects, the SMUP negative-peak amplitude for the modified method was 42±12 μV and for the standard method was 46±14 μV. At test 2, it was 40±9 for the modified method and 41±7 μV for the standard method. As for the elderly subjects, the SMUP negative peak amplitude was 45±11 μV for the modified technique and 43±7 μV for the standard technique. At test 2, the SMUP amplitude was 45±5 for the modified technique and 46±5 μV for the standard technique.

2. Motor Unit Number Estimation Results and Test-Retest Reliability:

In the younger subjects, the MUNE results obtained using the modified technique for test 1 and test 2 were 335±98 and 345±90 motor units, respectively. As for the standard MUNE method, it was 302±86 for test 1 and 332±57 for test 2. Test-retest reliability of the MUNE results obtained using the modified MPS technique (Cronbach's α=0.88) was substantially higher than the standard MPS technique (Cronbach's α=0.80; Figure 3-1). To test whether the increased test-retest reliability seen with the modified technique was due to a difference in the sample size of SMUPs obtained, we also analyzed the percentage errors of the MUNE when the same number of SMUPs was collected by the two methods. As described by Doherty and Brown (1993), the percent error represents the percentage difference between the motor unit number estimate derived with increasing numbers of SMUPs, used to calculate the average SMUP size and the MUNE derived using 11 SMUPs. Eleven, the maximum number of SMUPs, was deliberately chosen so that we could combine results from all the studies done in this paper for the analysis (Table 3-1). As shown in Figure 3-2, the mean percentage errors of MUNE at the same number of SMUPs are virtually identical, regardless which technique was used. This buttresses our argument that the increase in test-retest

reliability with the modified MPS technique was due to the larger sample of SMUPs obtained.

In comparison, in the elderly subjects, test-retest reliability of the MUNE results with both techniques was very high. Cronbach's α was 0.96 with the modified technique and 0.99 with the standard MPS technique. For the modified MPS method, the MUNE results was 226±85 motor units for test 1 and 220±73 motor units for test 2. For the standard MPS method, the MUNE results for test 1 and test 2 were 215±47 and 204±40 motor units, respectively.

The order of pulse width administration did not appear to affect the reliability of results. The MUNE test retest reliability in the last 6 subjects in whom the pulse widths were randomized, was the same as in the first 10 subjects where the short pulse width was administered first (Cronbach's α = 0.88 in both cases).

The additional use of 1 ms with the modified method took an extra 23±6 mins. That represented a 28% increase in the amount of time required.

3 – IV. Discussion:

In this study, we assessed the within-subject test-retest reliability of MUNEs obtained using a modified protocol of MPS. Standard MPS only uses a pulse width of 0.05 ms to obtain SMUPs, while this modified MPS used the combination of two pulse widths (0.05 ms and 1 ms). In the younger subjects, the test-retest reliability of the MUNEs was substantially improved by using the modified method, resulting in an increase of the Cronbach's α from 0.80 to 0.88. In contrast, the modified technique did not result in an improvement in the reliability of MUNE in the elderly subjects. However, this is perhaps not surprising since the test-retest reliability of the standard technique was already very high (Cronbach's α = 0.99) in the elderly subjects; it was impossible to increase the reliability any further. A likely reason for the substantially higher test-retest

reliability seen in the elderly subjects is that the SMUPs sampled as a percentage of the motor unit number in those individuals (10±3%) was significantly higher than in the younger subjects (7±3%) ($p<0.05$). The average SMUP thus derived in the elderly subjects was more likely to be representative of the total motor unit population. This suggestion is in keeping with results obtained by other investigators (Slawnych *et al.*, 1997;Felice, 1995).

The impact of sample size on the reliability of MUNE was also investigated by Doherty and Brown (1993). In that study, they showed that the MUNE percentage error declined substantially with a sample size of 10-15 SMUPs; a trend that we also observed with our dataset. However, if we only included the first 15 SMUPs obtained from each test session in this study, the test retest reliability would be substantially reduced, with a Cronbach's α of only 0.62. This supports the notion that an even larger SMUP sample size can indeed further increase the test-retest reliability of MUNE.

In this study, the median nerve innervated thenar MUNEs of 335±98 for the test, and 345±90 for the retest, in the young subjects and 226±85 for test 1 and 220±73 motor units in the elderly subjects, using the modified MPS method, were consistent with past studies using similar techniques conducted on the thenar muscles: 288±95 (Doherty & Brown, 1993) and 278±113 motor units (Wang & Delwaide, 1995). Since there is considerable variation in MUNE between healthy individuals, these minor differences are not unexpected (Simmons *et al.*, 2001).

Equally important when considering the clinical utility of this modified technique is that there was no apparent selection bias using the long duration stimulus pulse. Both amplitudes of the SMUPs and the MUNEs obtained using the two pulse widths were similar. What might the explanation be for the lack of sampling bias? A likely reason is that with the MPS technique, to collect the SMUPs, the median nerve is stimulated at a

large number of accessible sites throughout the wrist and the upper arm. Therefore, the cable properties of the motor axons are likely not to be the only factors that determine which SMUPs are activated. Factors that might be more important are the amount and density of interposed tissues and the relative depth of the motor axons in the fascicle at each location. Using a stimulation model, it was shown that preferential activation of large diameter motor axons is unlikely in MPS (Major & Jones, 2005). Additional SMUPs were selectively recruited when altering the stimulus pulse width, most likely because myelinated nerve fibers of different sizes have different thresholds (Grill, Jr. & Mortimer, 1996).

Based on our findings, the additional time needed for carrying out the modified MPS technique is worthwhile for younger subjects because it would substantially improve reliability of the test. In contrast, it is unnecessary in the elderly subjects who have reduced number of motor units.

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	Modified MPS # of SMUPs		Standard MPS # of SMUPs	
SUBJECT	TEST	RETEST	TEST	RETEST
1	23	21	16	17
2	24	19	20	17
3	30	21	24	16
4	25	27	14	20
5	16	26	11	22
6	25	25	21	22
7	18	20	15	19
8	16	16	13	15
9	24	26	21	19
10	22	25	16	19
11	26	23	22	17
12	19	15	15	11
13	19	23	16	18
14	17	22	13	14
15	21	23	17	19
16	22	21	17	18

Table 3-1. Comparisons of the number of surface motor unit action potentials (SMUPs) collected with the standard and modified MPS techniques during test and retest conducted on separate days. Subjects 1-11 are young and subjects 12-16 are elderly.

	Modified MPS SMUP Amplitude (μV)		Standard MPS SMUP Amplitude (μV)		Modified MPS MUNE		Standard MPS MUNE	
SUBJECT	TEST	RETEST	TEST	RETEST	TEST	RETEST	TEST	RETEST
1	38	32	44	33	433	443	365	425
2	64	50	72	53	190	288	168	280
3	31	31	33	36	427	403	389	342
4	38	37	45	42	330	352	270	310
5	48	43	56	49	344	343	291	296
6	59	61	69	42	171	181	147	266
7	36	36	40	38	463	430	400	410
8	26	32	25	28	365	275	385	327
9	38	41	40	45	400	388	348	377
10	48	45	44	42	249	234	281	257
11	34	35	38	40	315	455	279	361
12	40	47	36	41	138	130	160	153
13	63	52	54	54	152	176	173	172
14	34	39	38	42	342	292	268	248
15	48	44	45	45	225	204	226	216
16	40	43	42	46	271	298	250	233

Table 3-2. Comparisons of the mean surface motor unit action potential (SMUP) negative-peak amplitude, and motor unit number estimates (MUNE) during test and retest conducted on separate days. Subjects 1-11 are young and subjects 12-16 are elderly.

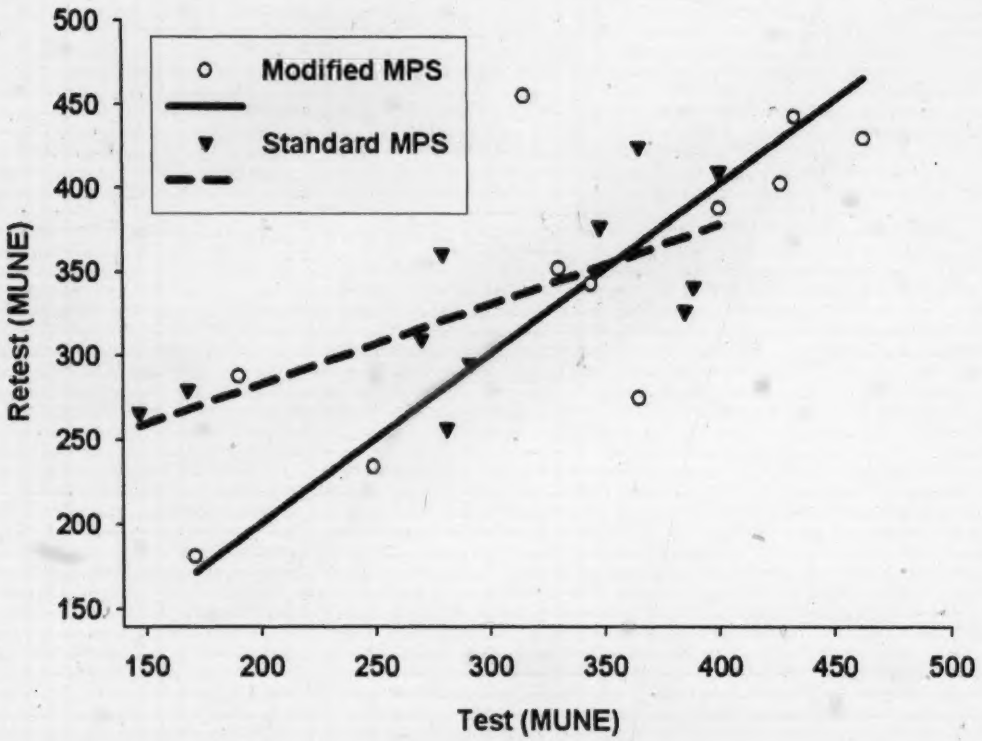

Figure 3-1. Reproducibility of the motor unit number estimation (MUNE), derived from the standard and modified multiple point stimulation (MPS) techniques, for each young subject. The black inverted triangles represent the standard MUNE technique and the open circles represent the modified MUNE technique. The dashed and solid lines represent the lines of regression for the standard and modified MUNE, respectively.

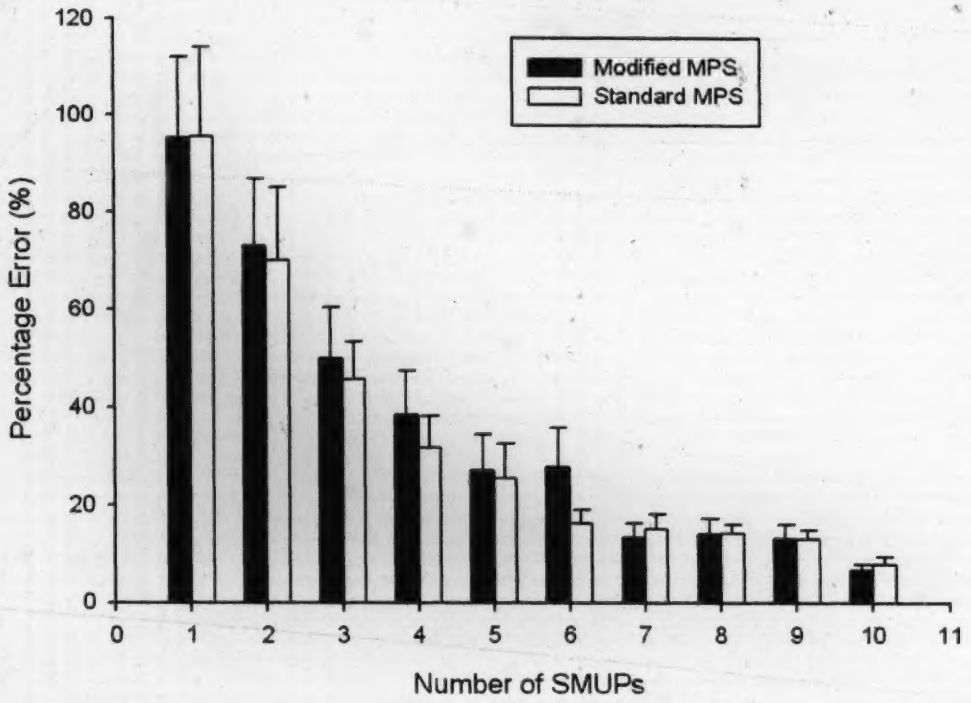

Figure 3-2. Percentage errors of motor unit number estimation (MUNE) obtained when progressively smaller numbers of surface motor unit action potentials (SMUPs) were used for the estimates. The percentage error was calculated by using the following equation: $|(MUNE^{1...11} - MUNE^{11})|$ / $MUNE^{11}$ x 100. Vertical bars denote the mean while the error bars denote standard error. At the same number of SMUPs, the percentage error was almost identical between the two methods.

CHAPTER 4

POST-SURGICAL ELECTRICAL STIMULATION FACILITATES AXONAL REGENERATION IN CARPAL TUNNEL SYNDROME

4 – I. Introduction:

Peripheral nerves have the ability to heal and regenerate (Seckel, 1990).This is in part because the Schwann cells (SCs) in the peripheral nervous system (PNS), unlike the oligodendrocytes in the central nervous system, support axonal regeneration (Ide, 1996). Although the PNS provides a more permissive environment for axonal regeneration, poor functional outcome often ensues (English, 2005; Sulaiman & Gordon, 2000). There are many reasons for poor axonal regeneration and functional outcome following peripheral nerve injury. These include a decline in the ability for axons to regenerate with distance and time (Boyd & Gordon, 2002), a progressive decline in the ability of the SCs to support axonal regeneration (Terenghi *et al.*, 1998), substantial delay of axons crossing the site of injury and repair (i.e. staggered regeneration; Al Majed *et al.*, 2000b), misdirection of the axons at the injury and repair site (Sunderland, 1978), axonal reinnervation of inappropriate targets (English, 2005) and muscle atrophy (Gutmann & Young, 1944). Due to the challenges of axonal regeneration and the fact that axonal regeneration is a slow process (axons regenerate approximately 1 – 3 mm/day in humans (Sunderland, 1978; Gutmann *et al.*, 1942), it is common that axons may never make functional contact with the appropriate muscles or sense organs in a timely manner, resulting in poor return of function.

Currently, there is neither a non-surgical nor surgical technique that ensures full functional recovery following peripheral nerve injury (Lundborg, 2003). For example, following surgery that alleviates the compression of the median nerve within the carpal tunnel (i.e, carpal

tunnel syndrome; CTS), axonal regeneration may still be unsuccessful and functional outcome may be suboptimal. In addition to the reduction in quality of life, poor axonal regeneration and functional outcome can result in high financial costs due to rehabilitation and loss of productivity attributed to disability leave. Therefore, it is important to investigate potential clinical interventions to enhance axonal regeneration and functional recovery and outcome following peripheral nerve injuries, such as CTS. CTS is also an appealing disease model to study because in addition to being a common condition, the location of nerve injury is identical in all subjects.

Various treatments have been examined, such as the application of neurotrophins (Boyd & Gordon, 2003), and thyroid hormones (Voria *et al.*, 2006). Electrical stimulation (ES) is a potentially useful clinical tool for improving axonal regeneration following peripheral nerve injuries. Studies on rats (Pockett & Gavin, 1985; Roman *et al.*, 1987; Al Majed *et al.*, 2000b; Mendonca *et al.*, 2003; Pomeranz *et al.*, 1984; Politis *et al.*, 1988), mice (Ahlborn *et al.*, 2007; Geremia *et al.*, 2007), rabbits (Nix & Hopf, 1983), canines (Rozman *et al.*, 2000), and humans (Amirjani, 2005) have shown that the application of low-frequency ES to crushed, compressed, or axotomized peripheral nerves enhances axonal regeneration.

For example, in one study, the effectiveness of ES on the axonal regeneration of rat femoral nerves was investigated (Al Majed *et al.*, 2000b). The femoral nerves were axotomized, resutured and ES (supramaximal pulses of 100 μsec; 3V, continuous 20 Hz train) was delivered proximally to the injured site for 1 h - 2 weeks by an implantable stimulator. Two weeks of ES enhanced the rate of axonal regeneration and improved the preferential growth of the axons to their appropriate end targets. More remarkably, only 1 h of ES produced similar results. The short duration of ES required for the positive effects on axonal regeneration made ES an even more appealing tool for clinical application.

In addition to enhancing axonal regeneration, ES has been shown to accelerate the rate of functional recovery in some animal studies (Nix & Hopf, 1983; Rozman *et al.*, 2000;

Mendonca *et al.*, 2003; Ahlborn *et al.*, 2007). For example, ES applied to transected mice femoral nerves accelerated the rate of functional recovery to near-maximum recovery 6 weeks earlier than controls (Ahlborn *et al.*, 2007). ES also appears to accelerate functional recovery of crushed nerves, as indicated by a faster recovery in the toe spread reflex in rats (Pockett & Gavin, 1985) and a quicker return to baseline measurements of tetanic tension, twitch forces and muscle action potentials in rabbits (Nix & Hopf, 1983). However, these findings are not universal. For example, in one study that examined functional outcome following the application of ES to crushed rat sciatic nerves (Mendonca *et al.*, 2003), both the stimulated and non-stimulated nerves showed a trend towards functional recovery. However, improvement in functional outcome in animals that were electrically stimulated compared to the controls, as indicated by the Sciatic Functional Index (SFI), was non-significant. The SFI is only an indirect measurement of the motor function of rat hind paws.

In the only human study on ES to date, although axonal regeneration was enhanced following the application of 1 h of direct ES (maximal tolerance limit of 10-20mA; 100ms duration and, continuous 20 Hz train) to the median nerves of subjects with moderate or severe CTS (Amirjani, 2005), functional outcomes between the treatment and control groups were not significantly different. In that study, electrodes were inserted intra-operatively on top of the nerve at the wrist where the nerve and surrounding tissues were anesthetized with a local anesthetics, lidocaine. That is potentially problematic as many sodium channels were blocked, preventing the ES from depolarizing all of the nerve fibers. Consequently, only a small fraction of the motor units in the thenar muscles could be activated.

To circumvent that difficulty, in this study, we depolarized the median nerve fibers more proximally at the elbow, well away from the site of the local anesthetics. By being able to depolarize a larger fraction of the median nerve fibers, we hypothesized that even more robust axonal regeneration and hence significantly better functional recovery would occur.

In this study, a monopolar needle electrode inserted percutaneously would be used. This carried an added advantage in that it is less invasive than the intraoperative wire electrodes used in the study conducted by Amirjani (2005).

4 – II. METHODS:

1. Overview of Methods:

To investigate if ES significantly improves motor axonal regeneration and functional outcome, a randomized controlled study was carried out on median nerves that had substantial axonal loss due to moderate or severe compression within the carpal tunnel. Immediately following CTS decompression surgery in the treatment group, low frequency ES was applied to the median nerve for 1 h at a proximal location to the injury site. The primary outcome measure was motor unit number estimation (MUNE), which evaluated the rate and extent of motor axonal regeneration and reinnervation to the median-innervated thenar muscles. Secondary outcome measures were used to evaluate subjective symptom severity, functional recovery and outcome. The secondary outcome measures were as follows: median motor and sensory nerve conduction studies (NC), Levine Self-Assessment Questionnaire (Levine), Semmes Weinstein Monofilaments test (SWM), Purdue Pegboard subtest (Purdue), and Moberg Pickup test (Moberg).

2. Subjects:

The procedures were carried out with the approval of the Human Ethics Board at the University of Alberta and the consent of the subjects. Seventeen subjects (3 males; 24 symptomatic hands) were recruited from an EMG clinic in Edmonton, Alberta. All subjects were right-hand dominant (the hand used for writing), except one subject in the control group. Seven subjects had severe bilateral CTS that decompression surgery was performed on both hands. Both hands were included in the study.

The selection of subjects was based upon clinical interviews, physical assessments and electrodiagnostic tests. Inclusion criteria required that the potential subject experienced at least one of the following symptoms: a) paresthesia and/or pain in the median nerve distribution of the hand, b) precipitated sensory disturbances by repetitive manual activities, c) nocturnal awakening due to sensory disturbances, d) weakness of thumb abduction, or d) atrophy of median-innervated thenar muscles. In addition to the presence of at least one of these symptoms, subjects were required to undergo electrophysiological tests that revealed moderate or severe median nerve compression within the carpal tunnel. NC studies were used to detect and classify the severity of CTS based upon electrodiagnostic criteria (Table 1-2; Padua et al., 1997). Subjects were considered to have moderate CTS when both the median sensory and motor CVs were abnormal across the carpal tunnel. Severe CTS was defined as when the SNAP was absent. To ensure that the hand symptoms were not due to other peripheral neuropathies, further electrophysiological tests were conducted on both the ulnar and radial nerves. To be included in the study, the subjects also must have undergone conservative treatments that did not alleviate the symptoms.

In our study, the exclusion criteria were as follows: a) the presence of additional medical or neurological conditions, b) other additional injuries to the symptomatic arm or hand, or c) presence of conduction block across the carpal tunnel.

3. Nerve Conduction Studies:

Both sensory and motor median NC studies were performed on the subjects using standard techniques on an Advantage EMG machine (Neurosoft Inc., Virgina; Dumitru, 1995a). A heat pack maintained the temperature of the symptomatic hand between 32 and 36 °C. The skin was gently abraded and cleansed with rubbing alcohol before disposable surface EMG strip electrodes (Ag/AgCl, 1 X 2.5 cm) were positioned. A ground electrode with electrolyte gel was

placed on the dorsum of the hand. All NC studies were performed with a hand-held bipolar constant current bar stimulator.

For the median sensory NC studies, an active electrode was positioned over the proximal interphalangeal joint of the third digit and a reference electrode was placed on the distal interphalangeal joint. The median nerve was stimulated supramaximally in the mid-palm and just proximal to the distal wrist crease to record the SNAP amplitude (negative peak). The sensory CV across the carpal tunnel was calculated.

For the median motor NC studies, an active electrode was positioned over the motor point of the thenar eminence muscles and a reference electrode was placed over the dorsal aspect of the first metacarpophalangeal joint. The median nerve was supramaximally-stimulated approximately 8 cm proximal to the active electrode and at the antecubital fossa. The maximal CMAP amplitude (peak-to-peak) and the terminal motor latency of the forearm were determined. To detect conduction block, the median nerve was supramaximally stimulated both proximal and distal to the carpal tunnel. A conduction block existed if there was a reduction in the maximal CMAP amplitude proximal to the carpal tunnel (Jillapalli *et al.*, 2003). If a conduction block was present the subject was excluded from the study because it is a major confounding factor to the interpretation of the primary outcome measure, MUNE (see section 2-III-1).

4. Treatment:

Subjects were assigned randomly to either the treatment or control group using a random number generator in Excel (Microsoft Inc, WA, USA). There were 12 symptomatic hands in the treatment group and 12 symptomatic hands in the control group. Subjects in both groups underwent CTS decompression surgery. An hour prior to surgery, subjects in the treatment group had EMLA cream (AstraZeneca Canada Inc, Ontario) applied on the skin over the antecubital fossa over the median nerve. The EMLA cream is a topical anesthetic (lidocaine 2.5 % and prilocaine 2.5 %) with limited penetration used for dermal analgesia (Juhlin *et al.*,

1980). It minimized the discomfort during needle insertion. The ES (stimulus intensity of 10 – 20 mA) was applied to the median nerve to subjects in the treatment group within 15 - 30 min following surgery.

5. Surgical Procedure:

CTS decompression surgeries were performed on the subjects by a plastic hand surgeon. Surgeries followed standard procedures of open CTS surgery (Rosenbaum & Ochoa, 1993), with the exception of one subject in the treatment group who underwent endoscopic CTS surgery. To minimize blood loss during open CTS surgery, a tourniquet was inflated on the supinated forearm of the subjects. Local anesthesia (lidocaine) was injected into the wrist, medial to the flexor carpi radialis tendon. A longitudinal incision (3 cm) was made from the distal wrist crease to the radial side of the forth digit. The TCL was transected along the ulnar side of the incision. Once the incision had been sutured, the tourniquet was deflated and the wrist bandaged with soft dressing.

For endoscopic CTS surgery, general anesthesia was used instead of localized anesthesia. A 1 cm transverse incision was made at the distal wrist crease in the middle of the wrist (centered over the palmaris longus (if present; Malhotra *et al.*, 2007). An endoscope (a thin flexible tube with a mounted light and camera) was then guided through the incision. The TCL was transected, the margins of skin were sutured, and the wrist was bandaged with soft dressing.

All subjects were asked to keep the operated arm elevated above the level of the heart for a couple of days to avoid swelling. Simple hand movements were encouraged. The dressing and sutures were removed 1- and 2- weeks post-operatively. Subjects remained off work for at least a month.

6. Electrical Stimulation of the Median Nerve:

Following surgery, subjects in the treatment group proceeded to a neurophysiology laboratory for the application of ES to the median nerve, while those in the control group received no further interventions. Approximately 15 – 30 min following surgery, subjects in the treatment group reclined with the operated hand placed in a stabilized elevated position. Disposable EMG surface recording electrodes (Ag/AgCl) were placed over the pronator teres muscle. The electrodes were then connected to an Advantage EMG machine (Neuroscan Inc, VA). The median nerve was supra-maximally stimulated at the antecubital fossa, where the EMLA cream was previously applied. The location of the stimulator, where the CMAP was elicited with the lowest intensity determined the best location for the insertion of a small 36 gauge monopolar needle electrode (25 mm). The needle electrode, connected to a portable electrical stimulator (model SD9, Grass Instruments, Providence, RI, USA), was inserted through the skin. The needle electrode was placed as close to the median nerve as possible by moving in small increments while stimulating the nerve. Advancement of the needle tip was stopped once a location with the lowest stimulus intensity requirement was reached. A plastic cuff with a broad base was used to stabilize the needle (Fig. 4-1). For 1 h, a 20 Hz train of continuous ES was applied at the highest tolerance intensity (10 – 20 V) with a 50 μs pulse width. The CMAP amplitude was monitored continuously throughout the session. Amplitude of the CMAP evoked by the indwelling needle electrode was compared to the maximal CMAP at baseline. The average extent of activation was 40±20% of the maximal CMAP. Both the frequency and duration parameters were selected based upon parameters of previous studies (Al Majed *et al.*, 2000b; Amirjani, 2005).

4 – III. Outcome Measures:

Outcome measures were used to assess the efficacy of ES on enhancing motor axonal regeneration, subjective symptom severity and functional recovery in the subjects. The primary

outcome measure was MUNE, which evaluated the rate and extent of motor axonal regeneration and reinnervation to median-innervated thenar muscles (Dorfman, 1990). Secondary outcome measures included: NC studies, Levine, SWM, Purdue and Moberg. NC studies were used to demonstrate the extent of focal compression of the median nerve within the carpal tunnel (Stevens, 1987). Levine was used to evaluate subjective symptom severity and the SWM was used to evaluate the sensory threshold of the median nerve-innervated digits. To assess functional outcome the performances on two hand function tests (i.e., Moberg and Purdue) were evaluated. Hand function tests were appropriate to assess both functional recovery and outcome because CTS produces both sensory and motor changes in the hand (Bell-Krotoski, 1991).

All outcome measures were conducted before and after surgery. The schedule of the post-operative (post-op) measurements was as follows: post-op1 at 2 - 5 months, post-op2 at 5 – 8 months, and post-op3 at 8 –19 months. The first post-op evaluation was chosen to be 2 – 5 months based upon the knowledge that an axon regenerates about 1 – 3 mm/day in humans, (Gutmann *et al.*, 1942; Sunderland, 1978) and the distance from the nerve injury to the thenar muscles is 70 - 80 mm (Amirjani, 2005). Therefore, by post-op1 some axonal regeneration, reinnervation and recovery could be expected to occur.

1. Motor Unit Number Estimation:

MUNE is an electrophysiological method that estimates the number of functional motor units in a muscle or group of muscles (Koc *et al.*, 2006). One of the MUNE techniques, known as multiple point stimulation (MPS), was used in this study to estimate the number of motor axons that regenerated and reinnervated the median-innervated thenar muscles (Dorfman, 1990). MUNE is known to be a reliable method for assessing neurogenic changes in CTS (Koc *et al.*, 2006). Brown first noted a significant decline in the estimated number of thenar motor

units in subjects with CTS (Brown, 1973). Upon reinnervation of the muscle the estimated number of motor units increases.

MPS was performed on an Advantage EMG machine (Neurosoft Inc., Virgina) using standard techniques, as described by Doherty and Brown (1993). The signals were band pass filtered between 5 Hz and 2 kHz and sampled at 5 kHz. The skin was gently abraded and cleansed with rubbing alcohol. Disposable surface EMG strip electrodes (Ag/AgCl, 1 x 2.5 cm) were used. The active electrode was positioned over the motor point of the median nerve-innervated thenar muscles. The reference electrode was placed over the metacarpophalangeal joint of the thumb with the ground electrode attached to the dorsum of the hand. To reduce movement artifacts, the thumb was immobilized with tape to the side of the palm in an adducted position. Hand temperature was maintained between 32 and 36°C using a heat pack.

The course of the median nerve was mapped, where it was most superficial (i.e., between the wrist and distal forearm, and from the antecubital fossa to the axilla) using a hand-held bipolar constant current stimulator (Fig. 4-2). Since in the upper arm the median nerve is in close proximity to the ulnar nerve, it was important to ensure that co-activation of the ulnar nerve was avoided by looking out for the following signs: 1) an initial positive deflection of the CMAP, 2) abduction of the fifth digit, and 3) electrical sensations in the fifth digit. When those signs were detected, the stimulator was moved anteriorly to another location.

The peak-to-peak amplitude of the maximal CMAP was determined by stimulating supramaximally 8 cm proximal to the distal wrist crease until the amplitude remained constant. Next, multiple points along the superficial median nerve were stimulated by finely graded threshold stimulation. At each location the stimulus intensity was increased until a surface motor unit action potential (SMUP) was evoked. SMUPs were defined as: all-or-none responses, occurring in a reproducible and orderly fashion, having no fractionation to identical stimuli, and being evoked with distinct thresholds (Chan *et al.*, 1998). The lowest threshold SMUP was collected using template subtraction. In other words, the all-or-none response (i.e. the SMUP)

was subtracted from the baseline. On occasion the next higher threshold SMUP was also obtained from the same location by subtracting it from the lowest threshold SMUP (recorded as baseline) via template subtraction. Once the lowest threshold SMUPs were collected, the stimulator was then moved approximately 15 mm along the median nerve to decrease the chance of activating previously collected SMUPs. Approximately 20 SMUPs were collected from each subject on the symptomatic side. The software temporally onset aligned the SMUPs and then averaged the SMUPs in a data-point-by-data-point manner. The MUNE was derived from dividing the peak-to-peak amplitude of the maximal CMAP by the average peak-to-peak SMUP amplitude. The average MPS estimate of motor units in the thenar muscles of healthy subjects without CTS (based on negative peak area) is 288 (± 95 SD; Doherty & Brown, 1993).

Conduction block can occur in CTS, and is a major confounding factor to the calculation of MUNE. Therefore, any patient found to have this was excluded from the study.

2. Median Nerve Conduction Studies:

NC studies were used to detect the extent of focal compression of the median nerve within the carpal tunnel. Both sensory and motor median NC studies were performed in the same manner as that used to detect and classify CTS (see section 2-II-3). The parameters measured were: a) the sensory nerve CV across the carpal tunnel, b) the SNAP (negative peak amplitude), c) the maximal CMAP (peak-to-peak amplitude), and d) the terminal motor latency.

3. Levine Self-Assessment Questionnaire:

Levine was specifically designed as an outcome measure for subjects with CTS (Changulani *et al.*, 2008). It is a validated tool with high internal consistency for monitoring symptom severity and functional status of subjects with CTS (Changulani *et al.*, 2008; Levine *et al.*, 1993). Levine consists of 2 parts: 1) the symptom severity scale and 2) the functional status scale (Appendix I). The symptom severity scale is composed of 11 items on a Likert scale,

which describes various symptoms associated with CTS, including: numbness, weakness, nocturnal symptoms, pain, and hand impairment. The score for each item ranged from 1 (no symptoms) - 5 (very severe symptoms). The functional status scale describes everyday activities that may be affected by CTS, such as holding a book, buttoning clothes, and opening jars. The score also ranged from 1 - 5 with 1 indicating no problem in performing the task and 5 indicating total inability to perform the task. Subjects were asked to fill out the questionnaire. If a subject had bilateral CTS, a separate questionnaire was completed for each hand. Results from both the symptom severity scale and the functional status scale were added. A total score of 95 denotes the most extreme severity of CTS while a score of 19 denotes a complete absence of symptoms.

4. Semmes Weinstein Monofilaments Test:

The Semmes Weinstein monofilaments test (SWM; Sammons Preston Roylan-Canada) is often used to diagnose sensory abnormalities, evaluate the distribution and degree of severity in sensory perception loss and follow progression after treatment (Table 4-1; Bell-Krotoski, 1992; MacDermid *et al.*, 1992; Szabo *et al.*, 1984). SWM is a sensibility test that is known to be very sensitive to sensory abnormalities in CTS subjects (Szabo *et al.*, 1984; Gellman *et al.*, 1986). In general, the earliest complaints of CTS are changes in the sensibility of the hand (Inglis *et al.*, 1972). To evaluate functional sensibility the test relies on the threshold of pressure perception of various parts of the hand, measuring the smallest amount of force required for detection (Dellon, 1990). A return to normal sensory threshold, first manifested as tingling and numbness, is the first neurophysiological sign of functional recovery following axonal regeneration (Dellon, 1990). In fact, sensory fibers not only become affected by nerve compression earlier than motor fibers (Lundborg *et al.*, 1982), but also take longer to recover (Inglis *et al.*, 1972). Therefore, a test that evaluates sensory perception loss, like SWM, should

detect the earliest onset of compression and follow the progression of the compression until it is eliminated (Szabo *et al.*, 1984).

There are many advantages for using SWM as an outcome measure, such as it: 1) discriminates by location, 2) measures threshold sensitivity (Levin *et al.*, 1978) provides more control than other hand-held instruments in managing application force variables, for example vibration and application amplitude (Bell-Krotoski & Tomancik, 1987), 4) is easy to administer, and 5) is portable.

The SWM consists of 20 nylon monofilaments (38 mm in length) that progressively increase in diameter and results in an increase in applied force (by the logarithm of base ten of the force in grams; Dellon, 1990). For example, the threshold difference between 2.83 and 4.93 filaments is not 2-, but 10-fold. Each monofilament is calibrated so it buckles on the surface of the skin when a maximal force is reached. The maximal force is maintained until the monofilament is released (Bell-Krotoski & Tomancik, 1987). The SWM does not quantify degrees of normality. It only discriminates between normal and abnormal cutaneous sensation (Bell-Krotoski & Tomancik, 1987; MacDermid *et al.*, 1994). Generally, the 2.83 filament (about 68 mg) is the index for normal threshold (Bell-Krotoski *et al.*, 1995). Therefore, any larger filament required for perception indicates sensory abnormality (Table 4-1).

In our study, standard techniques for SWM were employed (Callahan, 1984). The subjects placed their hands supinated on a table and closed their eyes. The subjects were told that a monofilament was to be applied anywhere on either hand. The subjects were asked to indicate when and where they were touched. The filaments were applied gently and randomly to the hands in ascending order until a filament was detected for each area of the hand. Each filament was applied in a uniform manner: perpendicular to the hand, from a height of 1 inch. Each filament remained on the skin for approximately 1.5 s (Weinstein, 1968). The filaments were applied and removed slowly to avoid providing an additional amount of applied force (Weinstein, 1968; Bell-Krotoski & Buford, Jr., 1997). The site of application was varied for both

hands. Three attempts were applied to each testing site to increase reliability (Bell-Krotoski & Buford, Jr., 1997). The filament had to be felt at least once at each site to count as sensory perception. The pressure felt only on the third digit, where sensory NC studies were also performed, was used for data analysis. The reasons for this were three-fold: 1) typically only the median nerve innervates the third digit (Wong *et al.*, 2006); 2) the third digit is thought to have a higher rate of sensory abnormality than other fingers in subjects with CTS (Silver *et al.*, 1985); and 3) if a subject knew only the third digit was being evaluated eventually it may lead to guessing when a filament was applied.

5. The Purdue Pegboard Test:

As sensibility is diminished in subjects with CTS, there is a concomitant loss of precision in hand functions (Thonnard *et al.*, 1999). Indeed, any sensory disturbance in the fingertips can compromise fine motor dexterity, such as picking up small objects (Schulz *et al.*, 1998). Purdue is a reliable and valid tool to assess hand dexterity in subjects with CTS (Amirjani, 2005; fess, 1986). Purdue (Model 32020, Lafayette Instrument Company, IN, USA) assesses fine manual dexterity (Haward & Griffin, 2001) by evaluating the ability and speed it takes for a subject to pick-up, manipulate, and insert small pegs into holes (Tiffin, 1968). Manual dexterity is indicated by the number of pegs placed into the holes within 30 s (Desrosiers *et al.*, 1995; Haward & Griffin, 2001). An increase in the number of pegs placed in the holes indicates an improvement in manual dexterity. Purdue has many advantages as an outcome measure: 1) easy to administer, 2) short duration (less than 10 min), 3) relatively low cost, and 4) portable (Haward & Griffin, 2001).

Purdue is comprised of a board with 4 cups across the top and 2 parallel vertical rows of 25 holes going down the center. The outside cups contained 25 pins, the second cup in from the right contained 20 collars, and the remaining cup had 40 washers. Subjects sat directly in front of the board. Typically the test is comprised of three subtests, but in our study only the first

subtest was used because it is the only subtest that assesses the function of the treated and non-treated hand separately. Improvement on the test using the non-treated hand would suggest improvement on the test with the treated hand is due to a practice effect.

Subjects were asked to pick-up one pin at a time with only their first three digits and place the pins in the holes down one column. The subjects were instructed to do so as quickly as possible within 30 s. Only the first three digits were used because typically the median nerve innervates only these digits and the ulnar nerve innervates the others (Wong *et al.*, 2006). Three trials were conducted on each hand, commencing with the dominant hand, which was determined by which hand was used for writing. Hands were alternated between trials. To provide the most reliable score, the test score was calculated by averaging the three trials (Buddenberg & Davis, 2000). The standard average number of holes filled is 17, with an overall range of 13 - 19 pegs (Reddon *et al.*, 1988).

6. The Moberg Pick-Up Test:

Moberg is a hand function test that evaluates functional awareness (e.g. locating small objects) and precision grip (Moberg, 1958). Precision grip refers to knowing how much pressure needs to be applied so an object can be picked up and will not inadvertently fall (Ng *et al.*, 1999). Moberg is a valid and reliable test for the evaluation of manual dexterity in subjects with CTS, for it assesses manual dexterity and is heavily influenced by digit sensitivity (Amirjani, 2005). It has advantages over other dexterity tests because it: 1) requires simple equipment (i.e., everyday objects), 2) assesses the dominant and non-dominant hand separately, 3) assesses sensory acuity, and 4) clearly demonstrates deficits.

Moberg consists of the following 12 objects: screw; nail; key; Canadian nickel and quarter; safety pin; washer; paperclip; three hexagonal nuts of different sizes (outer diameter of 1/2" and thickness of 1/4"; outer diameter of 1/2" and thickness of 1/8"; outer diameter of 3/8" and thickness of 1/8"); and wing nut (Fig.4-3A). The objects were spread randomly onto a

wooden platform (11.5 X 17.5 inch), on the same side as the hand to be tested. A round container (6-inch wide) was placed on the other side of the platform. The platform was placed length-wise and was used to maintain consistency over the extent of dispersion of the objects. Each subject was asked to pick-up one object at a time (with only their first three digits) and put each object into the container as quickly as possible. No sliding of an object was allowed. The dominant hand was tested first and thereafter the hands were tested alternately. The time taken was recorded with a stopwatch. Time began with the instruction of "Go" and stopped when the last object was placed in the container. After three trials of each hand the subject repeated the test, but this time with eyes closed (Fig. 4-3B). Typically, subjects compensate for the lack of cutaneous feedback by using visual cues to locate objects (Ng *et al.*, 1999). However, with eyes closed the subjects can only rely on the sensibility of the fingers to find the objects (Ng *et al.*, 1999). The procedure was the same as before, except the subjects held the container with the untested hand and were told when three objects remained. By indicating how many objects were left it was less likely subjects would try to remember the number of objects remaining and become distracted. The trials were averaged.

4 – IV. Statistical Analyses:

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc, Chicago, US). The significance level was set at $P \leq 0.050$ *a priori*. The Shapiro-Wilk Test of normality was used to determine if the tests were skewed from the Gaussian distribution. It indicated that all the results were significantly skewed. Therefore, non-parametric statistical tests were used for subsequent data analyses. Wilcoxon Signed Ranks tests were used to compare baseline measurements to each post-op within each group. The Mann-Whitney U tests were used to compare baselines, post-op1s (2 – 5 months), post-op2s (5 – 8 months) and post-op3s (8 – 19 months) of both treatment and control groups. Data were plotted as box-and-whisker plots. These plots represent the median (middle-line), the

25th and 75th percentiles (lower and upper margins of the box), and the 10th and 90th percentiles (lower and upper whiskers). Points outside of the whiskers represent outliers.

4 – V. Results:

Seventeen subjects with 24 symptomatic hands participated in this study. However, one subject (female, in the control group, with bilateral CTS) withdrew from the study due to other commitments that prevented follow-up measurements. Another subject (female, in the treatment group, with bilateral CTS) still had median nerve compression in one of her hands following surgery. Therefore that hand was excluded from the study. Data analyses were performed on 16 subjects (3 males, 21 hands) with 11 hands (8 subjects) and 10 hands (8 subjects) in the treatment and control groups, respectively. The average age was 50±17 years old for the treatment group and 62±12 years old for the control group.

In this study there were measurements of 21 symptomatic hands, but sometimes data could not be obtained from some patients at some of the post-op periods. The number of symptomatic hands included in each outcome measure at each post-op is shown in Table 4-2.

The average extent of motor unit pool activation by the needle electrode was 40±20% of the maximal CMAP.

4 – VI. Outcome Measures:

All the median values and ranges of each outcome measure at baseline and post-ops for the treatment group are shown in Table 4-3. The median values and ranges of each outcome measure at baseline and post-ops for the control group are shown in Table 4-4.

1. Motor Unit Number Estimation:

An increase in the estimated number of motor units indicates an increase in the number of motor axons that have regenerated and reinnervated a muscle or groups of muscles (Dorfman, 1990). The average number of motor units innervating the median-innervated thenar muscles in healthy subjects without CTS (using the MPS technique) is 288 ± 95 SD (Doherty & Brown, 1993). In our study, the median MUNE values were substantially lower at baseline with estimates of 145 and 149 motor units for the treatment and control groups, respectively (Fig. 4-4). Although in the treatment group there was an increase at post-op1, it did not reach statistical significance compared to baseline (P = 0.093). However, there were significant differences at post-op2 (P= 0.025) and post-op3 (P = 0.047) in comparison to baseline. In contrast, in the control group there were no significant differences found between baseline measurements and post-op1 (P = 0.326), post-op2 (P = 0.401), or post-op3 (P = 0.401). In terms of between group comparisons, there were no significant differences found between baselines (P = 0.573), post-op1s (P = 0.178), post-op2s (P = 0.115), or post-op3s (P = 0.090) of the treatment and control groups.

2. Median Nerve Conduction Studies:

The minimum median sensory CV cutoff across the carpal tunnel in healthy subjects is 50 m/s (Dumitru, 1995b). At baseline the median sensory CVs were well below healthy values: 28 m/s (range: 12 - 43 m/s) and 30 m/s (range: 25 - 44 m/s) for the treatment and control groups, respectively (Fig. 4-5). For the treatment group, with respect to baseline measurements, there was a significant improvement at post-op1 (P = 0.046), but neither at post-op2 (P = 0.593), nor post-op3 (P = 0.080). For the control group there was no significance at post-op1 (P = 0.500), post-op2 (P = 0.080), or post-op3 (P = 0.345) compared to baseline measurements. There was no significant difference in the sensory CVs between the baselines (P = 0.333), post-

op1s (P = 0.594), post-op2s (P = 0.749) and post-op3s (P = 0.165) of the treatment and control groups.

Typically, SNAP amplitudes are 10 – 20 μA (Quan & Bird, 1999). At baseline the median SNAP amplitude was 6 μA (range: 0 - 31 μA, 5 hands with no detectable SNAP) for the treatment group and 8 μA (range: 0 – 32 μA, 3 hands with no detectable SNAP) for the control group (Fig. 4-6). For the treatment group, in comparison to baseline measurements, there was a significant increase at post-op1 (P = 0.017), post-op2 (P = 0.028) and post-op3 (P = 0.005). Although phase cancellation caused by temporal dispersion of the SNAP is a potential confounding factor that can result in changes of the SNAP amplitude, this is not a likely explanation because the negative peak duration of the SNAP remain similar post surgically (1.8-1.9 ms) compared to baseline (1.5 ms).

In contrast, in the control group there was no significant difference between the baseline measurements and post-op1 (P = 0.866), post-op2 (P = 0.833) and post-op3 (P = 0.753). However, there were no significant differences when group comparisons were made at baseline (P = 0.556), post-op1s (P = 0.102), post-op2s (P = 0.958) and post-op3s (P = 0.626).

The upper-limit value for the terminal motor latency of the median nerve is 4.2 ms in healthy subjects without CTS (Dumitru, 1995b). In our study, the treatment group had a baseline median terminal motor latency of 7.1 ms (range: 4.1 – 10.4 ms) and the control group had a baseline median terminal motor latency of 5.4 ms (range: 3.5 – 6.3 ms; Fig 4-7). In the treatment group there was a significant difference at post-op1 (P = 0.005), post-op2 (0.012), and post-op3 (P = 0.005) with respect to baseline measurements. In the control group there was a significant difference at post-op1 (P = 0.015), post-op2 (P = 0.017), and post-op3 (P = 0.018) with respect to baseline measurements. There were no significant differences between the baseline measurements (P = 0.105), between the post-op1s (P = 0.346), post-op2s (P = 0.465), or the post-op3s (P = 0.767) of the control and treatment groups.

Typically, in healthy subjects the median CMAP amplitude is between 7 and 15 mV (Jackson & Clifford, 1989;Kraft & Halvorson, 1983). In our study, the baseline median CMAP amplitude was 7 mV (range: 1 - 15 mV) for the treatment group and 9 mV (range: 4 - 14 mV) for the control group (Fig. 4-8). For the treatment group, there were no significant changes following surgery: post-op1 ($P = 0.575$), post-op2 ($P = 0.401$) and post-op3 ($P = 0.093$). For the control group, there were also no significant differences at post-op1 ($P = 0.093$), post-op2 ($P = 0.110$), or post-op3 ($P = 0.463$) compared to baseline measurements. There were no significant differences between the treatment and control groups: baselines ($P = 0.382$), post-op1s ($P = 0.462$), post-op2s ($P = 0.211$), and post-op3s ($P = 0.495$).

3. Levine Self-Assessment Questionnaire:

Levine gauges how severe the subject perceives the CTS symptoms. The score on the questionnaire increases as the severity of CTS symptoms increases. A score of 19 on the Levine indicates the subject experiences no CTS symptoms, whereas a maximum score of 95 indicates the most severe CTS. The median scores at baseline were 53 (range: 42 - 79) and 53 (range 36 – 62) for the treatment and control groups, respectively (Fig. 4-9). In the treatment group there was a significant difference at post-op1 ($P = 0.005$), post-op2 ($P = 0.012$), and post-op3 ($P = 0.003$) compared to baseline measurements. In the control group there was a significant difference at post-op1 ($P = 0.008$), post-op2 ($P = 0.012$), and post-op3 ($P = 0.005$) with respect to baseline measurements. There was no significant difference between baseline measurements ($P = 0.500$), but there were significant differences between post-op1s ($P = 0.012$), post-op2s ($P = 0.002$), and post-op3s ($P = 0.001$) of the treatment and control groups. The treatment group had significantly lower median Levine scores than those of the control group. Four subjects experienced complete symptom relief (score of 19). These subjects were all in the treatment group.

4. Semmes Weinstein Monofilaments Test:

In the SWM, the first four filaments (marked as 1.65, 2.36, 2.44, 2.83) indicate normal sensory threshold perception. These filaments correspond to forces of 0.0045 g, 0.023 g, 0.0275 g, and 0.068 g, respectively. The filament marked 2.83 (approximately 0.068 g) is the index for normal threshold (Bell-Krotoski *et al.*, 1995). Therefore, any larger filament required for sensory perception indicates sensory abnormality, such as diminished light touch, diminished protective sensation, and loss of protective sensation (Table 4-1). Only the threshold of sensory perception for the third digit on the treated hand was assessed.

There was no significant difference between control and treatment groups. At baseline the median threshold of sensory perception was normal: 0.0275 g (range: 0.0045 g – 0.407 g) for the treatment group and 0.0275 g (range: 0.0045 g - 28.84 g) for the control group. There was no significant improvement at any post-op in the control or treatment groups. Although some subjects had reduced sensory perception, both groups (at all time points) had median values that indicated normal sensory perception thresholds.

5. The Purdue Pegboard Test:

The number of pegs placed into holes within 30 s on the Purdue was used to assess manual dexterity. The normal average score on the Purdue is 17, with a range of 13 - 19 pegs (Reddon *et al.*, 1988). At baseline the median score for the treated hand was 13 pegs for the treatment group and 12 pegs for the control group. The range was 7 - 17 pegs and 5 - 17 pegs for the treatment group and control group, respectively. Performing Purdue with the treated hand, the treatment group had significant differences at post-op1 ($P = 0.024$), post-op2 ($P = 0.048$), and post-op3 ($P < 0.001$) compared to baseline measurements (Fig. 4-10). In the control group there were also significant differences at post-op1 ($P = 0.022$), post-op2 ($P = 0.020$), and post-op3 ($P = 0.001$) compared to baseline measurements. There were no significant difference

between the two groups at baseline (P = 0.417), post-op1s (P = 0.078), post-op2s (P = 0.702) and post-op3s (P = 0.377).

The performance on the Purdue with the untreated hand was evaluated to ensure improvement was not due to a practice effect. At baseline the median score was 14 pegs for the treatment group and 13 pegs for the control group. The range was 8 – 17 pegs and 7 - 17 pegs for the treatment and control groups, respectively. For the treatment group, there was neither a significant difference at post-op1 (P = 0.569), nor at post-op2 (P = 0.191) compared to baseline measurements (Fig. 4-11). However, there was a significant difference at post-op3 compared to baseline measurements (P < 0.001). In the control group there was a significant difference at post-op3 (P = 0.016) compared to baseline measurements, but none at post-op1 (P = 0.168), nor post-op2 (P = 0.105). There were also no significant differences at baseline (P = 0.416), post-op1s (P = 0.329), post-op2s (P = 0.426), or post-op3s (P = 0.056) between the control and treatment groups.

6. The Moberg Pick-Up Test:

Moberg evaluates hand functionality by measuring the time required to pick up objects and place them into a container as fast as possible under two conditions: a) with eyes open and 2) with eyes closed. Performing the Moberg with the treated hand and eyes open, the median values at baseline were 15 s (range: 12 – 21 s) and 14 s (range: 11 – 36 s) for the treatment and control group, respectively (Fig. 4-12). For the treatment group, there was no significant difference at post-op1 (P = 0.078), post-op2 (P = 0.782), or post-op3 (P = 0.252) compared to baseline measurements. In contrast, for the control group, there were significant differences at post-op1 (P = 0.001), post-op2 (P = 0.010) and post-op3 (P = 0.005) compared to baseline measurements. There were no significant differences between baselines (P = 0.471), or post-op1s (P = 0.639) between the control and treatment groups. However, there were significant differences at post-op2s (P = 0.027) and post-op3s (P = 0.009).

Moberg was performed with the treated hand and eyes closed (Fig. 4-13). For the treatment group there was no significant difference at post-op1 ($P = 0.173$) compared to the baseline measurements, but there was a significant difference at post-op2 ($P = 0.006$) and post-op3 ($P = 0.017$). For the control group there were significant differences at post-op1 ($P = 0.042$), post-op2 ($P = 0.032$) and post-op3 ($P = 0.004$) compared to baseline measurements. There were no significant differences between baselines ($P = 0.340$), post-op1s ($P = 0.946$), post-op2s ($P = 0.828$), or post-op3s ($P = 0.113$) between the control and treatment groups.

Moberg was also performed using the untreated hand (with eyes open and with eyes closed) to ensure improvement was not due to a practice effect. In the treatment group, when Moberg was performed with the untreated hand and eyes open, there was no significant difference at post-op1 ($P = 0.775$) or post-op2 ($P = 0.582$) compared to baseline measurements (Fig. 4-14). However, there was a significant difference at post-op3 ($P = 0.014$). In the control group there was a significant difference between post-op1 and baseline ($P < 0.001$), but no significant differences at post-op2 ($P = 0.519$) or post-op3 ($P = 0.100$). Comparing the control and treatment groups, the baselines ($P = 0.031$), the post-op1s ($P = 0.004$) and post-op2s ($P = 0.047$) were significantly different. However, the post-op3s were not significantly different from one another ($P = 0.219$).

In the treatment group, performing the Moberg with the untreated hand and eyes closed, there were no significant differences between baseline and post-op1 ($P = 0.422$), or baseline and post-op2 ($P = 0.441$) or baseline and post-op3 ($P = 0.091$) measurements (Fig. 4-15). In the control group, there was no significant difference at post-op1 ($P = 0.190$) or post-op3 ($P = 0.537$) compared to baseline measurements. However, there was a significant difference at post-op2 ($P = 0.011$). In comparing the control and treatment groups, there were no significant differences between baselines ($P = 0.276$), post-op1s ($P = 0.431$), post-op2s ($P = 0.112$), or post-op3s ($P = 0.367$).

4 – VII. Discussion:

In this study, we assessed the effectiveness of the application of 1 h of post-surgical ES on axonal regeneration in subjects with extensive axonal loss due to moderate and severe CTS. ES was found to be a promising adjunct clinical tool for treatment. Equally important, it was well tolerated by the subjects and resulted in no complications.

1. Effectiveness of ES on Axonal Injury:

a. Motor Axonal Regeneration:

The primary outcome measure used to assess the effectiveness of ES on motor axonal regeneration was MUNE. In subjects with extensive axonal loss due to moderate and severe CTS, the application of 1 h of post-surgical ES over the median nerve at the elbow significantly increased the estimated number of motor units of the median-innervated thenar muscles (Table 4-3). In contrast in the control group, CTS decompression surgery alone did not significantly improve the MUNE (Table 4-4). Therefore, the results of this study suggest that ES enhances motor axonal regeneration. However, there was no significant difference in MUNE change following intervention between the treatment and control groups. The most likely reason is the wide distribution of MUNEs in each group (Fig. 4-4; Tables 4-3 and 4-4). This large variability within the data consequently decreased the power of the statistical analysis to detect a significant difference between the treatment and control groups. Independent group comparisons are much less powerful than paired comparisons. Based on a sample size power analysis, [using the equation: $n = 2[PI \times \sigma/(\mu_1 - \mu_2)]^2$, whereas the n denotes the number of subjects required in each of the groups, PI denotes the power index (which depends on the stringency of alpha and beta), σ denotes the standard deviation, and $\mu_2 - \mu_1$ denotes the

treatment effect (the difference in means between the groups), a sample size of 25 in each group was required to have sufficient power to detect any differences between the groups. Therefore, in this study, the sample size was underpowered. Although there was no significant difference between groups detected in this study, it is important to note that overall there was a trend of significant difference of MUNE.

The results of this study, as well as those reported by others (Pomeranz *et al.*, 1984; Roman *et al.*, 1987; Nix & Hopf, 1983; Politis *et al.*, 1988; Rozman *et al.*, 2000;Mendonca *et al.*, 2003; Amirjani, 2005; Al Majed 2000; Brushart, 2002), show that ES significantly enhances motor axonal regeneration. Conversely, the MUNE results are in disagreement with those found in a study conducted by Pockett and Gavin (1985). In that study ES was applied to the crushed sciatic nerves of rats for 25 min - 1 h (Pockett & Gavin, 1985). ES appeared to significantly accelerate axonal regeneration, but without significantly increasing the number of motor axons that reinnervated the muscle. The discrepancy between the MUNE results could be due to the method used to assess the number of motor units within the muscle in that study. In the study conducted by Pockett and Gavin (1985) the nerve was stimulated proximal to the lesion with 0.1 ms pulses, which increased gradually in voltage. An electrode was placed on the muscle near the end-plates and the EMG traces were observed on an oscilloscope. A correspondence between an increase in stimulation voltage and an increase in EMG represented one motor unit. In our study, MUNE was the method used to assess the number of motor units that had reinnverated the thenar muscles. MUNE is known to be a useful, reliable method, unlike the method used in the former study.

In a study conducted by Amirjani (2005), ES was applied to the median nerve at the wrist following CTS decompression surgery. Although lidocaine from the local anesthesia blocked some of the sodium channels at the wrist and potentially inhibited some of the motor axons from depolarizing, ES resulted in a significant increase in MUNE in the treatment group at post-op2 and post-op3 compared to baseline measurements. No significant differences were

found in the control group. In our study, ES was applied more proximal (i.e., away from the sodium blockade) and MUNE also improved at post-op2 and post-op3 in the treatment group with no improvement in MUNE in the control group. Therefore, depolarization of more motor axons did not produce a further significant improvement in MUNE. Perhaps, ES exerts its effects on the soma, but there is a ceiling effect, whereby additional depolarization of the motor axons does not further improve the effects of ES on motor axonal regeneration and reinnervation.

Compared to MUNE, the CMAP is not very sensitive to early reinnervation by motor axons. This was shown in this study, with the CMAP amplitude not significantly improving even though there was a significant improvement in MUNE. This may be explained by the fact that the CMAP amplitude is based on the constituent sizes and configurations of SMUPs (Felice, 1997). In early reinnervation the SMUPs are small and polyphasic (Dorfman, 1990). These SMUPs do not contribute much to the CMAP amplitude for much phase cancellation can occur amongst the SMUPs. Conversely, collateral sprouting may have masked the loss of motor axons at baseline. Our results agree with those of Amirjani (2005), which also showed a lack of significant improvement in CMAP amplitudes following CTS decompression surgery and the application of ES.

The terminal motor latency was measured to determine if the conduction speed improved with intervention. There were significant improvement at all post-op periods in the treatment group, and in the control group. The improvement seen in the treatment group is in agreement with the results of Amirjani (2005). In that study the treatment group demonstrated significant improvement in the terminal motor latency at all post-ops, while there was no significant improvement in the control group at any post-ops.

b. Sensory Axonal Regeneration:

To evaluate the effectiveness of ES on sensory axonal regeneration of the large sensory fibers that subserve vibration and proprioception, the SNAP amplitudes were measured. The median SNAP amplitudes significantly increased following the application of ES at all post-ops. In contrast, in the control group there was no significant improvement in the SNAP amplitude. At baseline the median SNAP amplitudes at 6 and 8μV for the treatment and control group were below the lower limit of normal (10 μV). The lack of significant difference between the groups may be due to the wide range of SNAP amplitudes.

Similar to the results found in our study there was a significant improvement in SNAP amplitude in the treatment group in the study conducted by Amirjani (2005). The study concluded that ES accelerated the recovery of the SNAP amplitude, significantly improving earlier in the treatment group (at post-op2 compared to baseline measurements) than in the control group, which significantly improved at post-op3. In our study there was no significant improvement in the SNAP amplitudes within the control group.

The sensory CV was expected to significantly improve in both groups following surgery. In our study there was no significant difference between the treatment and control groups at any post-ops. In the treatment group post-op1 was significantly different than baseline measurements ($P = 0.046$), while there were no significant differences at post-op2 ($P = 0.593$) or at post-op3 ($P = 0.80$). In the control group there were no significant differences at post-op1 ($P = 0.500$), at post-op2 ($P = 0.080$) or at post-op3 ($P = 0.345$).

The SWM test was used to evaluate the small sensory fibers of the median nerve, which detect pressure and touch. There was neither a significant improvement in the sensory perception threshold in the treatment, nor in the control group. Contrary to the findings in this study, the sensory perception thresholds significantly improved in both the treatment and control groups in a similar study (Amirjani, 2005). The discrepancy between these studies may be

attributed to the fact that in our study the median thresholds of sensory perception were normal at baselines for both the treatment and control groups. Therefore, no further improvement was possible.

It is not surprising that at baselines the large sensory nerve fibers appear to have been severely damaged but not the small sensory nerve fibers. This concurs with the pathophysiology of nerve compression whereby the large diameter fibers are affected by the compression before the small fibers.

2. Clinical Implications:

Both the treatment and control groups showed significant improvement at all post-ops as indicated by lower Levine scores. This is perhaps not surprising, as decompression surgery is known to alleviate median nerve compression symptoms (Katz *et al.*, 1998). However, it is important to note that the treatment group had significantly lower Levine scores than those of the control group at all post-ops. The four subjects that recovered completely from subjective CTS symptoms all belonged to the treatment group. These results agree with a study conducted by Amirjani (2005) that also demonstrated that the subjective CTS symptoms significantly improved in the treatment group versus the control group, as measured by Levine.

Although the subjective symptoms improved, hand-function dexterity, as measured by the Purdue Pegboard Test and the Moberg Pickup Test, did not. However, it is important to note that hand dexterity is strongly influenced by the subjects' ability to perceive touch. Since touch sensitivity of the hand in these subjects was normal even at baseline and that it did not change with surgery, the lack of change in hand function performance is not unexpected. Another possible reason for no significant improvement could be due to the wide variability within the data and the small sample size.

These results are in accordance with another human study that also found that functional recovery did not improve with ES (Amirjani, 2005).

3. Fraction of Motor Neuron Pool Stimulated for Electrical Stimulation Effects:

In a previous human study ES was applied to the median nerve at the wrist (Amirjani, 2005). However, in that study there was a sodium channel blockade (due to the local anesthesia) at the wrist. Therefore, only a small fraction of the motor axon could be depolarized. In contrast, in our study ES was applied more proximally at the antecubital fossa away from the potential sodium blockade. Although we suspected that because more nerve fibers were depolarized, this would result in further significant improvement in motor axonal regeneration and functional outcome and recovery. However, this did not occur. Indeed, the results of the two studies were very similar. This unexpected finding raises an important issue: perhaps only depolarization of a fraction of motor axons is needed to significantly enhance motor axonal regeneration. This is practically important consideration as subjects are more willing to tolerate low intensity stimulation. This would increase the feasibility of large scale clinical implementation of post surgical electrical stimulation. However, to provide a definitive answer to this question, further experiments that systematically evaluate the impact of different stimulus intensity are needed.

4 – VIII. Potential Mechanisms of Action of Electrical Stimulation:

The expression of injury/regeneration-associated genes is increased following the application of ES to an injured nerve (Al Majed *et al.*, 2000b; Al Majed *et al.*, 2004). This suggests that ES mediates its positive effects on axonal regeneration at the soma. Additional evidence for this notion is that tetrodotoxin (which blocks retrograde action potential transmission from the periphery to the soma) eliminates the positive effects of ES on axonal regeneration (Al Majed *et al.*, 2000a; Al Majed *et al.*, 2000b). ES may augment the response of the soma by up-regulating the substrates required for facilitating axonal growth (Geremia *et al.*, 2007). At the molecular level the following changes have been observed following the

application of ES to an injured nerve: 1) very rapid up-regulation of brain-derived neurotrophic factor (BDNF) and expression of its high affinity receptor (trk B; Al Majed et al., 2000a; English, 2005); 2) up-regulation of Tα1-tubulin (Al Majed *et al.*, 2004); 3) up-regulation of GAP-43 (Al Majed *et al.*, 2004); 4) down-regulation of medium-molecular weight neurofilament protein (NFM; Al Majed *et al.*, 2004); and 5) increase in neuronal cyclic adenosine monophosphate (cAMP) levels (Hempel *et al.*, 1996).

BDNF is a candidate for enhancing regeneration of motor axons (Al Majed *et al.*, 2000b; Boyd & Gordon, 2002). BDNF and neurotrophin-4/5 (NT-4/5) are required for the regeneration of motor axons (English, 2005). There is an up-regulation of BDNF mRNA and its receptors (i.e, trkB and p75) in both axotomized motor neurons and in denervated SCs (Al Majed *et al.*, 2000a; Boyd & Gordon, 2002) 3 days following injury (Frostick *et al.*, 1998). Therefore, ES is considered to exert part of its effects by up-regulating BDNF and its receptor trk B (Al Majed *et al.*, 2000a; English, 2005). This is strongly supported by evidence that links electrical activity and BDNF expression in both *in vitro* (Ghosh *et al.*, 1994) and *in vivo* studies (Castren *et al.*, 1992) and 2) *in situ* hybridization studies of motor neurons that demonstrate early, dramatic up-regulation of BDNF and trk B in response to ES (Al Majed *et al.*, 2000a). In addition, BDNF and trk B mRNA were detected at low levels in intact rat femoral motor neurons, but increased following axotomy (Al Majed *et al.*, 2000a).

The up-regulation of BDNF and trk B is most likely related to both calcium and cAMP (Brushart *et al.*, 2002). ES facilitates calcium influx, which depolarizes the axon. The axon must be depolarized for ES to promote axonal regeneration (Ming *et al.*, 2001). *In vitro* studies have shown that calcium enters the cell body, via depolarization, and this leads to an up-regulation of immediate early genes, initiation of gene expression and neurite outgrowth (Kocsis *et al.*, 1994).

Besides BDNF and its trk B receptor, ES is thought to also regulate the expression of GAP-43, Tα1-tubulin, and NFM (Al Majed *et al.*, 2004). The application of 1 h of ES to

axotomized rat femoral nerves causes an increase in both GAP-43 and Tα1-tubulin mRNA levels, and a decrease in NFM mRNA levels (Al Majed *et al.*, 2004). These changes in gene expression are associated with the roles of these factors in axonal regeneration. For example, immediately ensuing injury, GAP-43 (an axonally transported phosphoprotein) is up-regulated by about 100 times within the membrane of regenerating axons and returns to its normal concentration upon reinnervation (Skene *et al.*, 1986; Fawcett & Keynes, 1990). GAP-43 appears to play an essential role in axonal growth. GAP-43 is known to have a role in spontaneous axonal sprouting (Aigner & Caroni, 1995). The simultaneous up-regulation of Tα1-tubulin and down-regulation of NFM mRNA levels possibly augment axoplasmic transport by increasing the fluidity of the axoplasm (Doherty *et al.*, 1995), providing faster transport of tubulin along the axon, and consequently faster axonal regeneration than if there was no up-regulation of Tα1-tubulin and down-regulation of NFM mRNA levels (Hoffman *et al.*, 1985).

ES might also enhance axonal regeneration by compressing the time it takes for axons to start regenerating across the injury site (Al Majed *et al.*, 2000b) In other words, ES might compress staggered regeneration temporally across the injury site, thereby enhancing axonal regeneration (Al Majed *et al.*, 2000b; Brushart, 1988 ;Geremia *et al.*, 2007). It remains uncertain if ES would drive dormant neurons into the regenerative process quicker or if ES alters the regenerative program (Brushart, 1988; Geremia *et al.*, 2007). ES has also been shown to decrease axonal misdirection at the injury/repair site, thus increasing the probability that the regenerating nerve reinnervates its appropriate end target (e.g., preferential motor reinnervation) (Al Majed *et al.*, 2000b; English, 2005).

4 – X. Limitations and Future Directions:

1. Subject:

One hand in the treatment group underwent endoscopic carpal tunnel release instead of open carpal tunnel release surgery. The endoscopic technique is claimed to result in less pain, and has a shorter recovery period than the open technique (Trumble *et al.*, 2002). However, evidence is lacking. One study indicated that endoscopic surgery resulted in less post-operative pain and less limitation of activity than open surgery (Atroshi *et al.*, 2006). However, the differences between the results were small (Atroshi *et al.*, 2006). In that study, it was concluded that hands that underwent endoscopic surgery experienced improvement in symptoms faster than those that underwent open carpal tunnel release, but the results at 6 months between the open and endoscopic technique were similar. In the long-term, results of endoscopic release surgery are the same as open release surgery (Brief & Brief, 2000). In other words, there is a similar degree of symptom relief and improvement of the subjects between techniques (Atroshi *et al.*, 2006). In our study it was more important to improve the long-term effects (post-op3) than the short-term effects (post-op1). Therefore, although there could have been differences in the short-term between hands undergoing different procedures, the hand of this subject remained in the study. The hand that underwent endoscopic surgery had similar results as the rest of the hands that underwent open surgery.

2. Experimental Procedures:

While having the same experimenter performing both the ES (immediately after surgery) as well as the outcome tests simplifies the study and reduces the experimenter error, it does not allow the study to be blinded. Therefore, biasness could occur. Future studies should be double-blinded, that is one person should perform the ES procedure and a second person should conduct all outcome tests and analyses without knowing whether or not the subject received ES treatment.

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Filament Calibration	Force Applied	Severity in Sensory Perception Loss
1.65 2.36 2.44 2.83	4.5 – 67.7 mg	Normal sensory threshold
3.22 3.61	166 – 408.2 mg	Reduced light touch
3. 4. 4.31	695.8 – 2052 mg	Reduced protective sensation
4.56 – 6.65	3632 – 447000 mg	Loss of protective sensation

Table 4-1. The calibration (i.e. markings) of the Semmes Weinstein monofilaments, force applied, and degree of severity in sensory perception loss.

Outcomes	Treatment Group (Hands)				Control Group (Hands)			
	Baseline	P-1	P-2	P-3	Baseline	P-1	P-2	P-3
MUNE	11	9	8	11	10	8	8	8
Sensory CV	6	10	7	10	6	8	7	9
SNAP	11	10	7	10	9	9	9	7
CMAP	11	10	8	10	10	9	9	7
TML	11	10	8	10	10	9	9	7
Levine	11	10	8	11	10	9	8	10
SWM	11	10	11	10	10	9	9	9
Purdue	11	10	8	11	10	8	9	9
Moberg	8	7	8	11	9	8	9	9

Table 4-2. The number of symptomatic hands included in each outcome measure at baseline and at each post-op. Post-op1 represents 2 -5 months, post-op2 represents 5-8 months, and post-op3 represents 8- 13 months post-surgery. MUNE is the motor unit number estimation, sensory CV is the sensory conduction velocity, SNAP is the sensory nerve action potential amplitude, CMAP is the compound muscle action potential, TML is the terminal motor latency, and SWM is the Semmes Weinstein Monofilaments test. Overall there were 11 and 10 symptomatic hands in the treatment and control groups, respectively. Symptomatic hands were excluded if the subject could not perform the test or if there was not enough time to perform the test.

Outcomes (units)	Treatment Group			
	Baseline	Postop-1	Postop-2	Postop-3
MUNE (motor units)	145 (71-533)	219 (104-473)	225 (157- 626)	221 (130-589)
Sensory CV (m/s)	28 (12-43)	37 (22-46)	36 (22-45)	35 (30-45)
SNAP (μA)	6 (0-31)	17 (1-29)	7 (3-41)	13 (1-32)
CMAP (mV)	7 (1-15)	8 (6-10)	8 (8-10)	9 (6-10)
TML (ms)	7.1 (4.1-10.4)	4.5 (3.9-5.3)	4.2 (3.8-4.6)	4.1 (3.6-4.5)
Levine	53 (42-79)	23 (19-27)	21 (19-25)	20 (19-25)
SWM (g)	0.0275 (0.0045-0.407)	0.0275 (0.0045-0.166)	0.0275 (0.0045-0.166)	0.0275 (0.0045-0.692)
Purdue Tx (pegs)	13 (7-17)	14 (8-17)	14 (7-17)	15 (8-18)
Purdue Untx (pegs)	14 (8-17)	14 (9-17)	14 (10-17)	15 (10-18)
Moberg Tx Eyes Open(s)	15 (12-21)	13 (10-24)	14 (11-32)	14 (10-25)
Moberg Tx Eyes Closed(s)	31 (17-49)	26 (21-40)	24 (19-39)	26 (19-39)
Moberg Untx Eyes Open (s)	15 (11-22)	15 (10-25)	15 (12-20)	14 (10-23)
Moberg Untx Eyes Closed(s)	27 (18-47)	26 (19-40)	25 (21-32)	25 (19-40)

Table 4-3. Median values of the outcome measures in the treatment group at baseline and at each post-op. Ranges of the median values are shown in brackets. Post-op1 represents 2 -5 months, post-op2 represents 5-8 months, and post-op3 represents 8- 13 months post-surgery. MUNE is the motor unit number estimation, sensory CV is the sensory conduction velocity, SNAP is the sensory nerve action potential amplitude, CMAP is the compound muscle action potential, TML is the terminal motor latency, and SWM is the Semmes Weinstein Monofilaments test. Tx = treated hand, Untx = untreated hand.

Outcomes (units)	Control Group			
	Baseline	Postop-1	Postop-2	Postop-3
MUNE (motor units)	149 (14-217)	168 (28-224)	165 (128-266)	160 (117-300)
Sensory CV (m/s)	30 (25-44)	32 (28-41)	35 (30-37)	38 (32-57)
SNAP (μA)	8 (0-32)	9 (0-12)	9 (5-11)	8 (6-23)
CMAP (mV)	9 (1-14)	7 (2-10)	7 (5-11)	7 (5-11)
TML (ms)	5.4 (3.5-6.3)	4.1 (3.8-6.0)	3.9 (3.5-4.8)	3.9 (3.8 – 5.0)
Levine	53 (36-62)	27 (22-44)	28 (24-34)	27 (20-42)
SWM (g)	0.0275 (0.0045-28.84)	0.0275 (0.0045-0.166)	0.0680 (0.0275 – 0.407)	0.0275 (0.0045 – 0.166)
Purdue Tx (pegs)	12 (5-17)	12 (7-18)	13 (8-17)	14 (9-18)
Purdue Untx (pegs)	13 (7-17)	13 (6-18)	13 (9-17)	13 (9-19)
Moberg Tx Eyes Open(s)	14 (11-36)	13 (9-20)	12 (9-20)	12 (9-21)
Moberg Tx Eyes Closed(s)	27 (20-62)	27 (19-46)	25 (16-29)	24 (15-26)
Moberg Untx Eyes Open(s)	13 (11-26)	13 (9-22)	13 (9-20)	13 (8-18)
Moberg Untx Eyes Closed(s)	26 (19-40)	25 (17-33)	27 (17-42)	24 (16-31)

Table 4-4. Median values of the outcome measures in the control group at baseline and at each post-op. Ranges of the median values are shown in brackets. Post-op1 represents 2 -5 months, post-op2 represents 5-8 months, and post-op3 represents 8- 13 months post-surgery. MUNE is the motor unit number estimation, sensory CV is the sensory conduction velocity, SNAP is the sensory nerve action potential amplitude, CMAP is the compound muscle action potential, TML is the terminal motor latency, and SWM is the Semmes Weinstein Monofilaments test. Tx = treated hand, Untx = untreated hand.

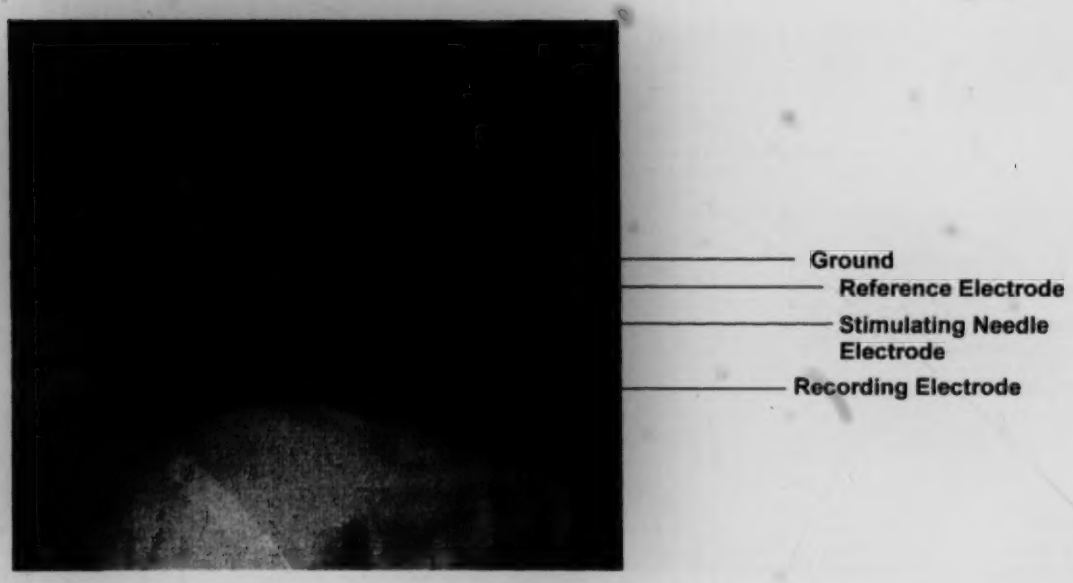

Figure 4.1. Experimental Set-Up. Set-up of electrodes during electrical stimulation.

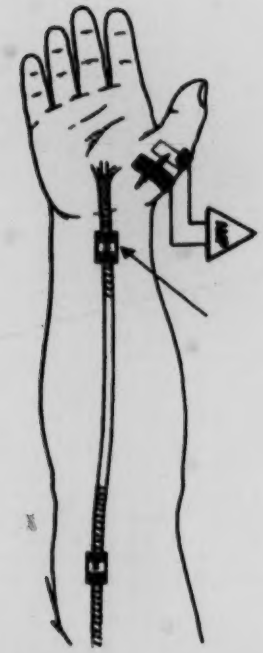

Figure 4-2. Set-up of the multiple point stimulation technique.

(A) (B)

Figure 4-3. Moberg Pickup Test. In Fig. (A) the 12 objects that comprise the Moberg Pick-up Test are depicted. In Fig. (B) a subject is performing the Moberg Pickup Test with his eyes closed.

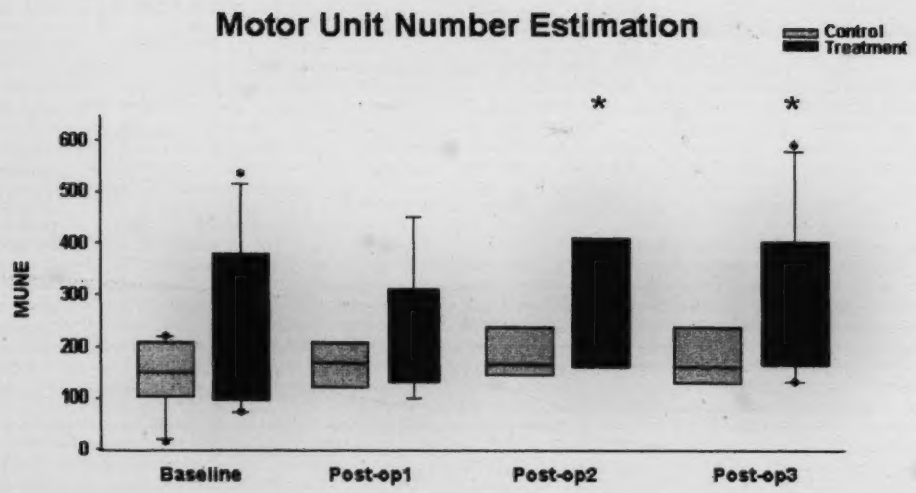

Figure 4-4. Comparison of MUNE in the control and treatment groups. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline.

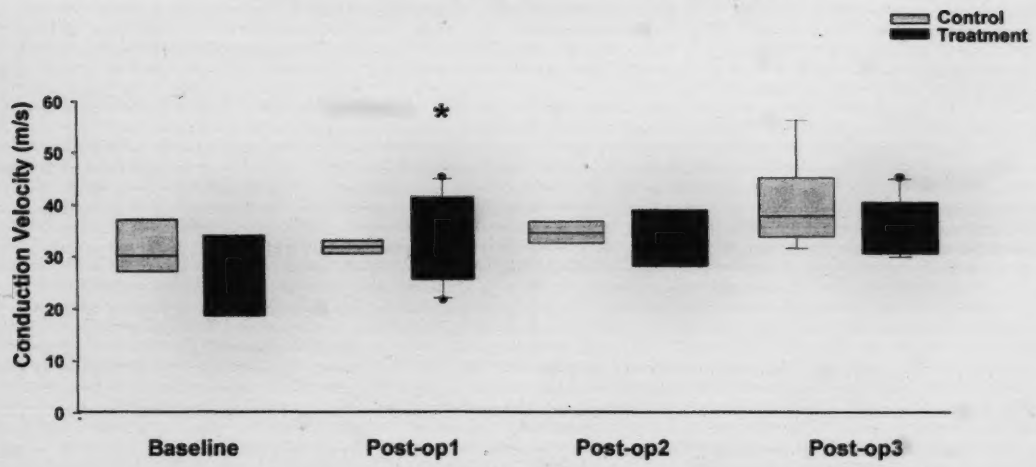

Figure 4-5. Sensory nerve conduction velocity. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline.

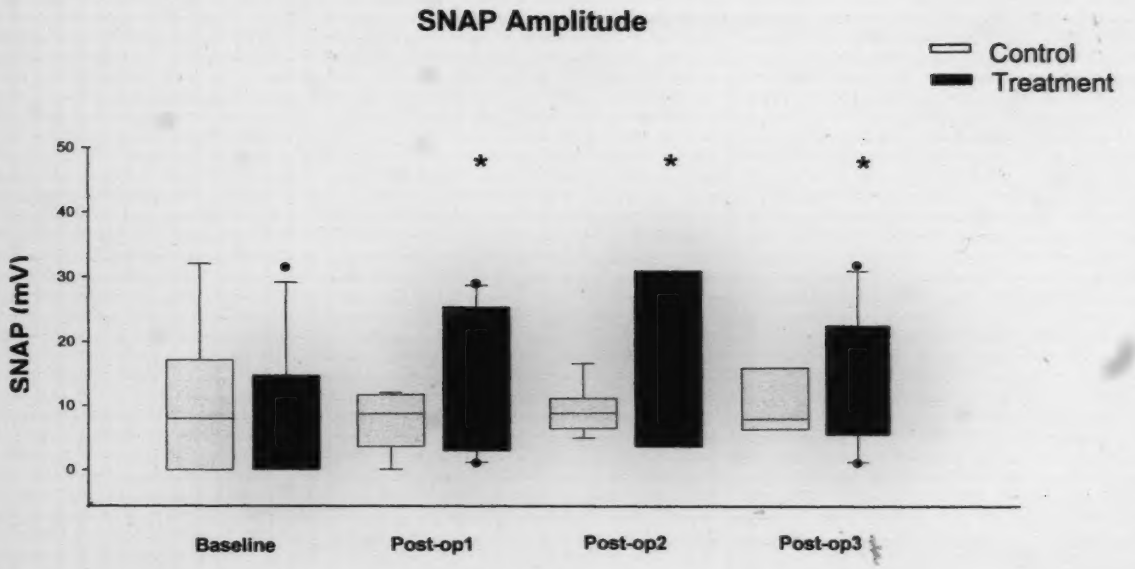

Figure 4-6. Sensory nerve action potential amplitude (SNAP). Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline.

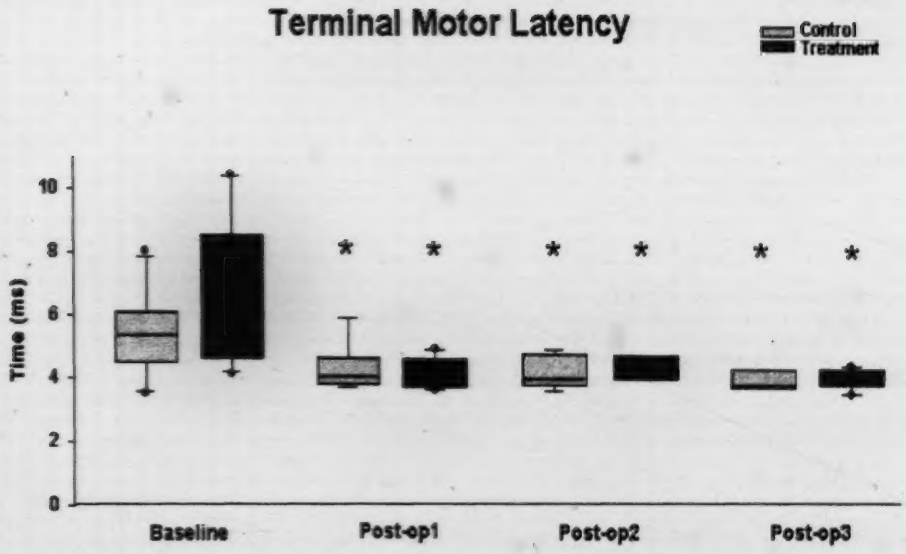

Figure 4-7. Terminal motor latency. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline.

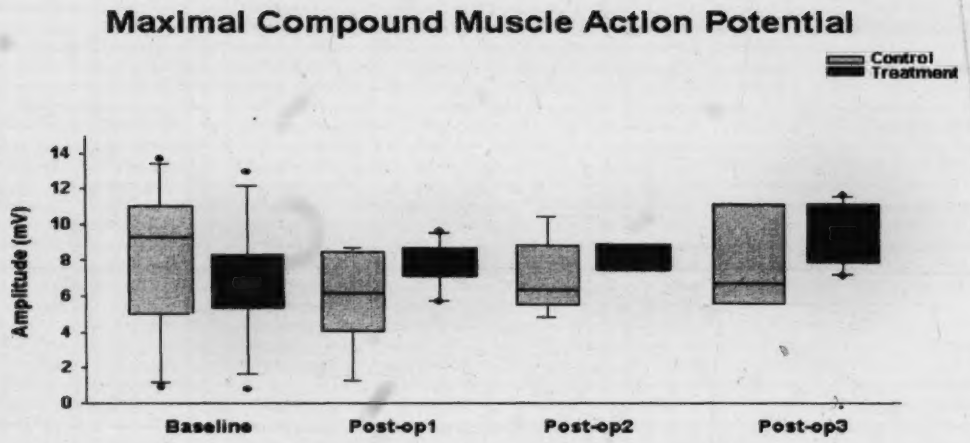

Figure 4-8. Compound muscle action potential amplitude. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers.

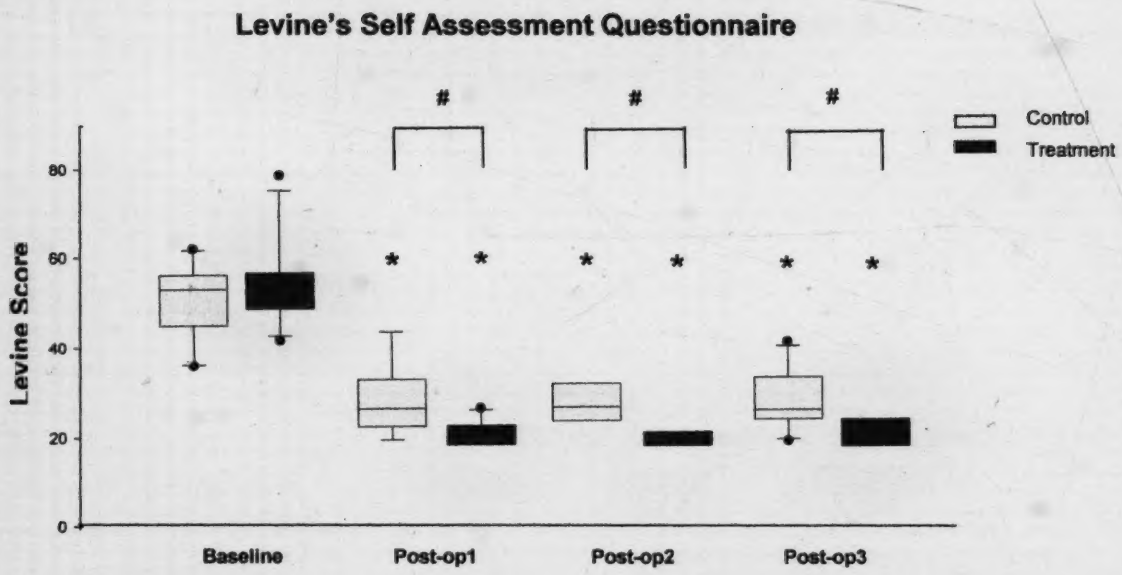

Figure 4-9. Comparison of Levine's Self Assessment Questionnaire. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline. # denotes a significant difference ($p < 0.05$) in comparison between the control and treatment groups.

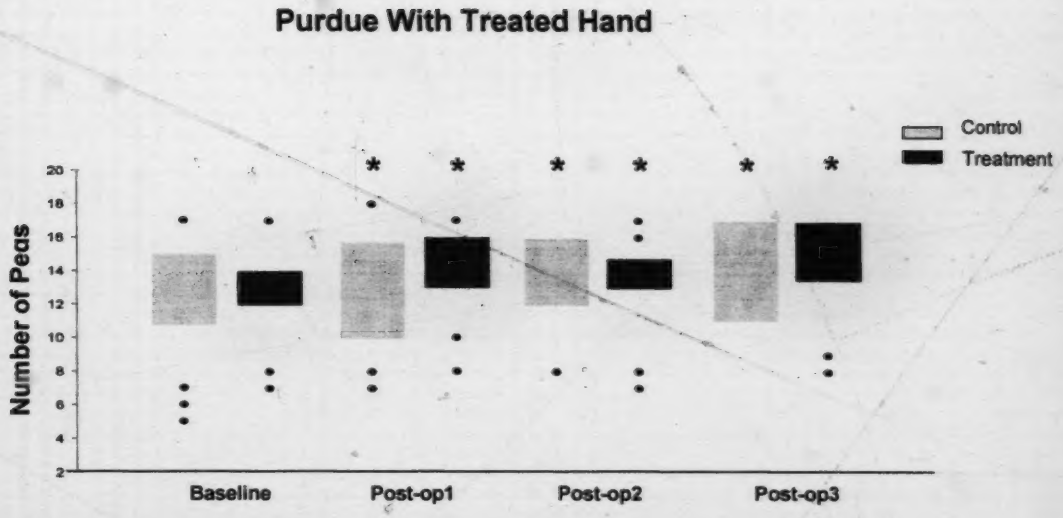

Figure 4-10. Comparison of the performance on the Purdue Pegboard test using the treated hand. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline.

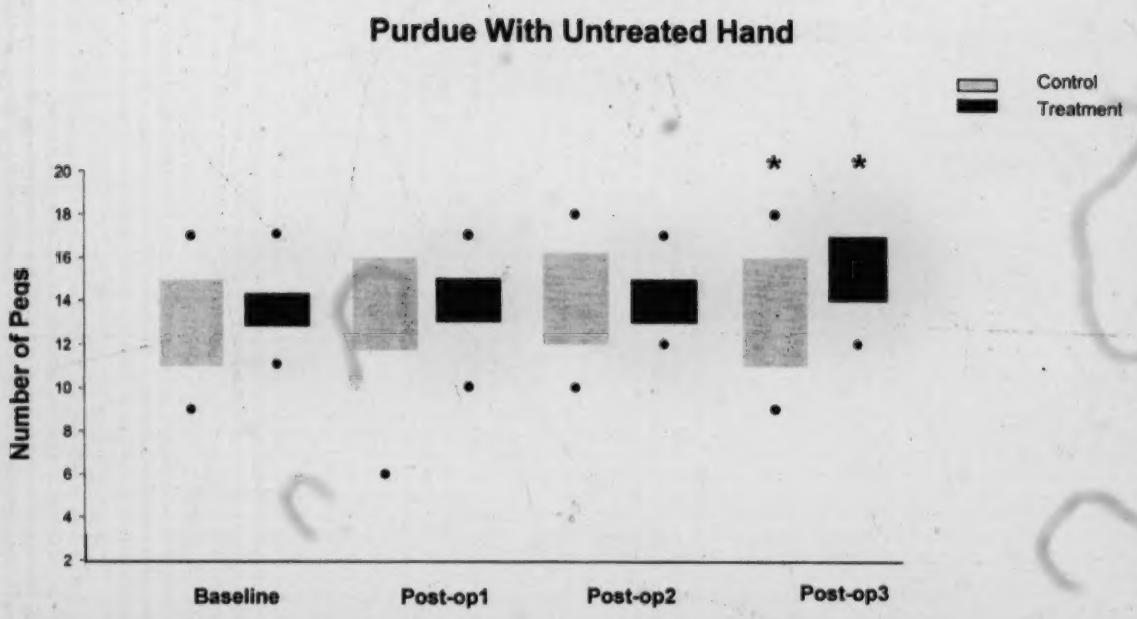

Figure 4-11. Comparison of the performance on the Purdue Pegboard Test using the untreated hand. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline.

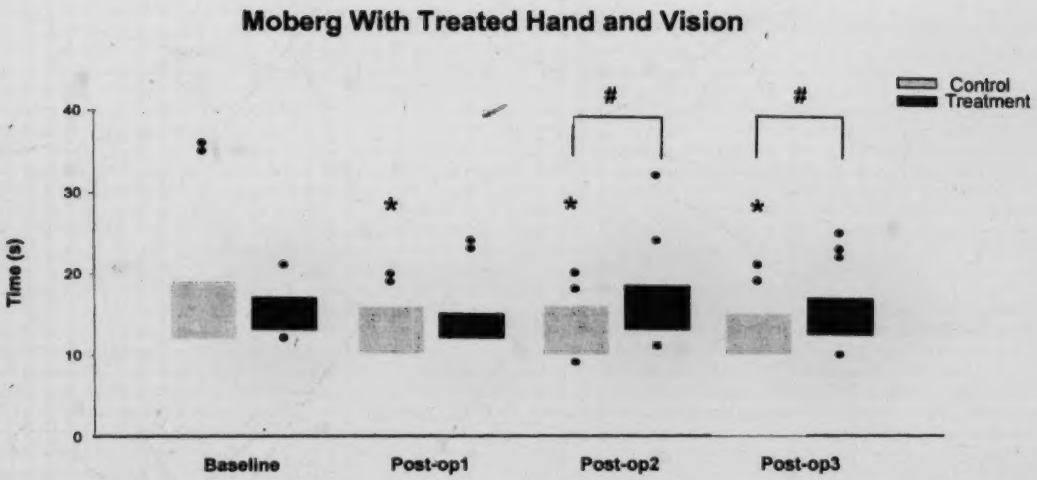

Figure 4-12. Performance on the Moberg Pickup Test using the treated hand with eyes open. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline. # denotes a significant difference ($p < 0.05$) in comparison between the control and treatment groups.

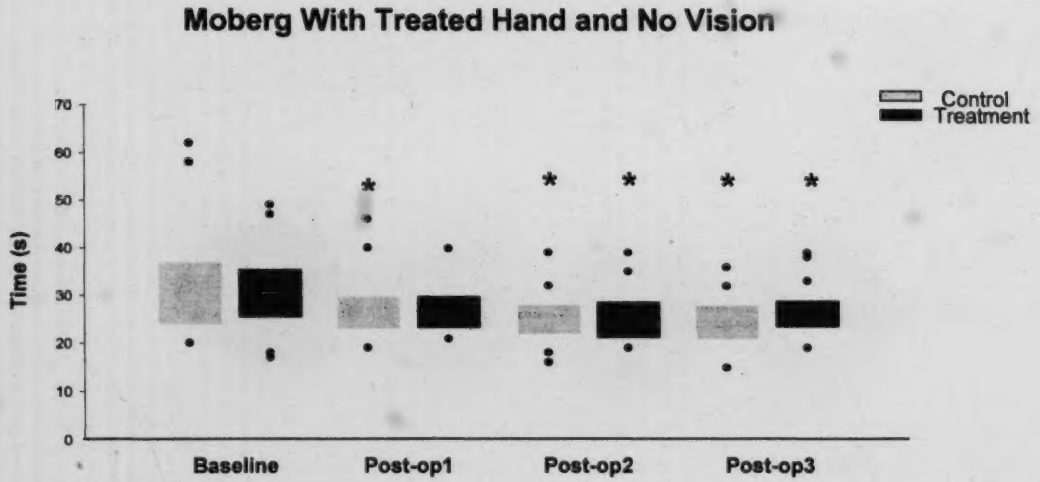

Figure 4-13. Performance on the Moberg Pickup Test using the treated hand with eyes closed. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline.

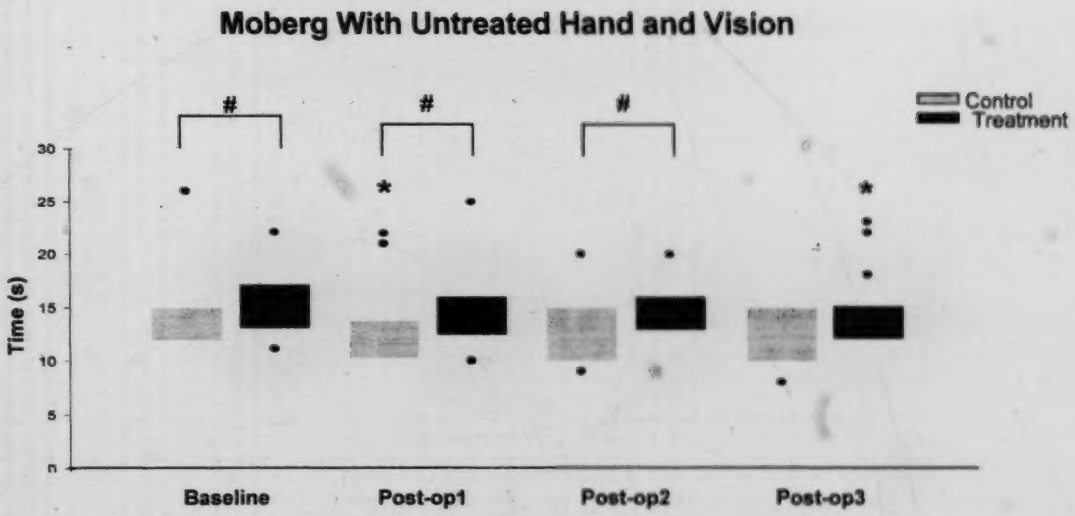

Figure 4-14. Performance on the Moberg Pickup Test using the untreated hand with eyes open. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline. # denotes a significant difference ($p < 0.05$) in comparison between the control and treatment groups.

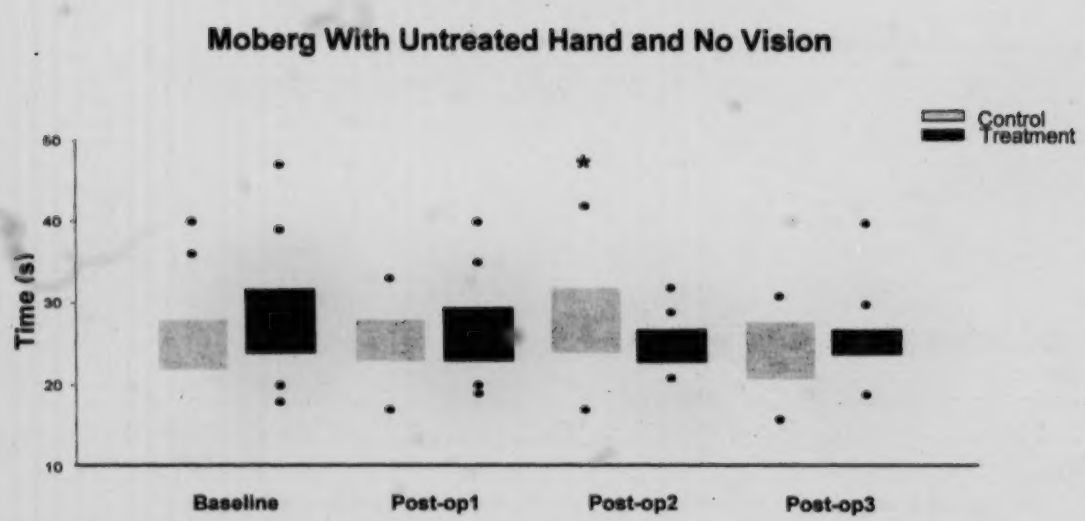

Figure 4-15. Performance on the Moberg Pickup' test using the untreated hand with eyes closed. Box plots represent the median (middle line), the 25th and 75th percentiles (lower and upper margins of the box) and the 10th and 90th percentiles (lower and upper whiskers). Black dots outside the whiskers represent outliers. * denotes $p < 0.05$ in comparison with baseline.

CHAPTER 5

CONCLUSION AND FUTURE DIRECTIONS

Given the importance of finding a reliable means of estimating the number of motor units in axonal injuries, one of the initial goals of my thesis was to derive a method to improve the test-retest reliability of MUNE. We chose to focus on the MPS method because it is well suited for studying the median nerve and that it is also one of the most widely used methods. To address the critical issue of finding ways to enhance motor nerve regeneration, the second major goal of this thesis was to test the effects of ES on axonal regeneration of the median nerve, functional recovery and outcome in human subjects with substantial axonal loss due to moderate or severe CTS. To achieve this goal 1 h of ES was applied to the median nerve immediately following CTS decompression surgery and outcome measures were assessed.

5– I. Improving the Reliability of the Multiple Point Stimulation Technique:

We found that the additional use of long stimulus pulse width did increase the sample size of single motor unit action potentials. Although potential mechanisms have been postulated by other investigators using computer models, they have not been tested by direct experiment to test whether those theoretical assumptions are indeed correct. This step would be crucial and should be addressed in future experiments. One potential method of approach is by systematically modulating the excitability of individual motor axons by manipulating their resting

membrane potentials. It would be instructive to compare the order of activation in response to different pulse widths. If there turns out to be systematic differences in the state of polarization of the axolemmal membrane potential to different pulse width stimulus, the role of ion channels may be further elucidated by pharmacological means in reduced nerve preparations.

It was gratifying to find that the modified MPS technique did result in an improvement of the test-retest reliability of younger subjects. Based on that observation, we are now beginning to implement a large scale study to test the practicality of this method in routine clinical settings on patients with different peripheral nerve conditions.

5 – II. Effectiveness of Electrical Stimulation in Enhancing Axonal Regeneration and Functional Outcome:

My results show that 1 h of low-frequency ES, applied immediately following CTS decompression surgery is adequate to significantly enhance axonal regeneration, but not functional outcome. This is the first study that investigated the effectiveness of applying ES proximal to a nerve injury site in human subjects with CTS (i.e., depolarizing all the motor nerve fibers) on enhancing axonal regeneration and functional outcome. It suggests that the depolarizing more motor axons does not result in a further significant increase in axonal regeneration, or a significant improvement in functional outcome.

Our results suggest that ES is promising as a clinical tool for peripheral nerve injuries, for it reduces the delay of axons crossing the injury site (i.e. staggered regeneration). An enhancement in axonal regeneration is one step closer to improving functional outcome because to have a chance for successful function, axons have to regenerate and reach appropriate end targets in a timely manner. ES is also a promising clinical tool for: only 1 h of ES is required to produce the positive effects on axonal regeneration; the application of ES is a relatively non-invasive procedure; and most importantly ES is tolerable for human subjects.

5 – III. Effectiveness of Using a Longer Pulse Width to Improve Multiple Point Stimulation:

My results show that the reliability of multiple point stimulation technique can be improved by using a long stimulus pulse width (1 ms), in addition to the standard 0.05 ms stimulus pulse width. There was no selection bias using the long stimulus pulse width. Both amplitudes of the SMUPs and the MUNEs obtained using the two pulse widths were similar. The results suggest that a larger SMUP sample size can further increase the test-retest reliability of MUNE. This greater test-retest reliability should enhance the usefulness of MPS as an outcome measure in clinical studies.

5 – IV. Future Directions for the Effectiveness of Electrical Stimulation:

The effects of ES on functional outcome should be further explored by using additional functional outcome measures. Typically, those with CTS lose hand strength. Therefore, future studies should include maximal voluntary hand strength as an outcome measure, which can be tested by using a Jamar dynamometer. A Jamar dynamometer measures the grip strength of those with hand trauma and dysfunction. Also tentanic force of the median-innervated thenar muscles should be measured to evaluate muscle fatigue, for ES was shown to significantly improve MUNE, indicating that motor axons reinnervated the thenar muscles, making effective nerve-muscle connections (Doherty *et al.*, 1995).

In this study only 1 h of ES was shown to significantly accelerate motor axonal regeneration and recovery. However, future studies should examine the possibility of decreasing the duration of the ES and still attaining the positive ES effects. Some studies have supported this notion (Wood & Willits, 2006; Pockett & Gavin, 1985). For example, *in vitro* study on dorsal root ganglia of chick embryos, 0.40 – 0.50 mA of uniform electrical fields was applied for only 10 mins (Wood & Willits, 2006). The brief ES significantly enhanced neurite outgrowth, as it did for longer duration studies of 1 h (Jaffe & Poo, 1979; Patel & Poo, 1982). *In vivo* study

on crushed rat sciatic nerves, the application of 5 or 10 min of ES immediately following injury resulted in a significant improvement in functional recovery of the toe-spreading reflex (Pockett & Gavin, 1985).

ES has been shown to significantly enhance the early onset of axonal regeneration by compressing staggered regeneration. In the future, ES should be combined with modalities that accelerate and prolong axonal outgrowth (Brushart *et al.*, 2002). For example, ES could be used to rapidly initiate the regenerative process and recruit the motor axons across the repair site, followed by the application of neurotrophins that may significantly increase the speed and extent of motor axonal regeneration (Brushart *et al.*, 2002).

Our study suggests that post-surgical ES enhances the motor axonal regeneration in subjects with moderate or severe CTS. Such a nerve injury is defined as axonotmesis. This extent of nerve damage is not as severe as a transection (also known as neurotmesis). Therefore, in the future it is important to investigate if ES could enhance axonal regeneration in a transected nerve, or in a more proximal nerve injury (e.g., ulnar nerve or median nerve compression at the Guyon's canal at the antecubital fossa). Typically in these cases, successful axonal regeneration and return in function are less common. In neurotmesis the endoneurial tube is disrupted and axons are more apt to be misdirected at the nerve injury site. Since ES has previously been shown to enhance preferential motor reinnervation (Al Majed *et al.*, 2000) perhaps ES can facilitate axonal regeneration in transected peripheral nerves. It has also be suggested that ES compresses staggered regeneration temporally, thus enhancing axonal regeneration (Brushart *et al.*, 2002). Timing is important in proximal peripheral nerve injuries because the axon needs to regenerate a longer distance than more distal nerve injuries and there is a decline in the capacity of axons to regenerate with time and distance.

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Appendix:

Levine's Self-Assessment Questionnaire for Carpal Tunnel Syndrome

Subject ID# _____ **Date** _____

The following questions refer to your symptoms for a typical twenty-four (24) hour period during the past two weeks (circle one answer to each question).

A. How severe is the hand or wrist pain that you have at night?

1. I do not have hand or wrist pain at night.
2. Mild pain
3. Moderate pain
4. Severe pain
5. Very severe pain

B. How often did hand or wrist pain wake you up during a typical night in the past two (2) weeks?

1. Never
2. Once
3. Two or three times
4. Four or five times
5. More than five times

C. Do you typically have pain in your hand or wrist during the daytime?

1. I never have pain during the day
2. I have mild pain during the day
3. I have moderate pain during the day
4. I have severe pain during the day
5. I have very severe pain during the day

D. How often do you have hand or wrist pain during the daytime?

1. Never
2. Once or twice a day
3. Three to five times a day
4. More than five times a day
5. The pain is constant

E. How long, on average, does an episode of pain last during the daytime?

1. I never get pain during the day
2. Less than 10 minutes
3. 10 to 60 minutes
4. Greater than 60 minutes
5. The pain is constant throughout the day

F. Do you have numbness (loss of sensation) in your hand?

1. No
2. I have mild numbness
3. I have moderate numbness
4. I have severe numbness
5. I have very severe numbness

G. Do you have weakness in your hand or wrist?

1. No weakness
2. Mild weakness
3. Moderate weakness
4. Severe weakness
5. Very severe weakness

H. Do you have tingling sensation in your hand?

1. No tingling
2. Mild tingling
3. Moderate tingling
4. Severe tingling
5. Very severe tingling

I. How severe is numbness (loss of sensation) or tingling at night?

1. I have no numbness or tingling at night
2. Mild
3. Moderate
4. Severe
5. Very severe

J. How often did hand numbness or tingling wake you up during a typical night during the past two (2) weeks?

1. Never
2. Once
3. Two or three times
4. Four or five times
5. More than five times

K. Do you have difficulty with the grasping and use of objects such as keys or pens?

1. No difficulty
2. Mild difficulty
3. Moderate difficulty
4. Severe difficulty
5. Very severe difficulty

Functional Status Scale

On a typical day during the past two weeks have hand and wrist symptoms caused you to have any difficulty doing activities listed below? Please circle the one number that best describes your ability to do the activity.

Activity	No Difficulty	Mild Difficulty	Moderate Difficulty	Severe Difficulty	Cannot do at all due to hand or wrist symptoms
Writing	1	2	3	4	5
Buttoning of clothes	1	2	3	4	5
Holding a book while reading	1	2	3	4	5
Gripping of a telephone handle	1	2	3	4	5
Opening of jars	1	2	3	4	5
Household chores	1	2	3	4	5
Carrying of Grocery Bags	1	2	3	4	5
Bath and dressing	1	2	3	4	5

From Levine, D et al., A self-administered questionnaire for the assessment of severity of symptoms and functional status in carpal tunnel syndrome. *Journal of Bone and Joint Sugery.* Vol 75-A, No. 11, November, 1993, Pg. 1585.